IOBC / WPRS and IOBC / EPRS

Working Groups
"Insect Pathogens and Insect Parasitic Nematodes"

Les Groupes de Travail
"Les Entomopathogènes et Nématodes Parasites d'insectes"

Proceedings of the first joint meeting

Poznan (Poland)
27 August - 1 September 1995

Edited by

P.H. Smits

IOBC / wprs Bulletin
Bulletin OILB / srop Vol. 19(9)1996

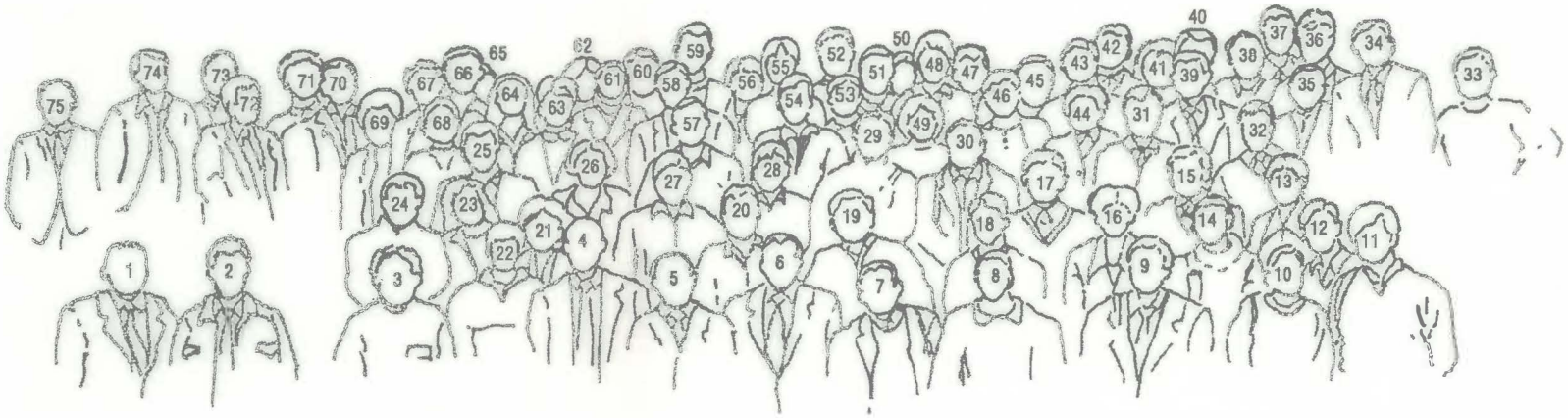
The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section (IOBC/WPRS)

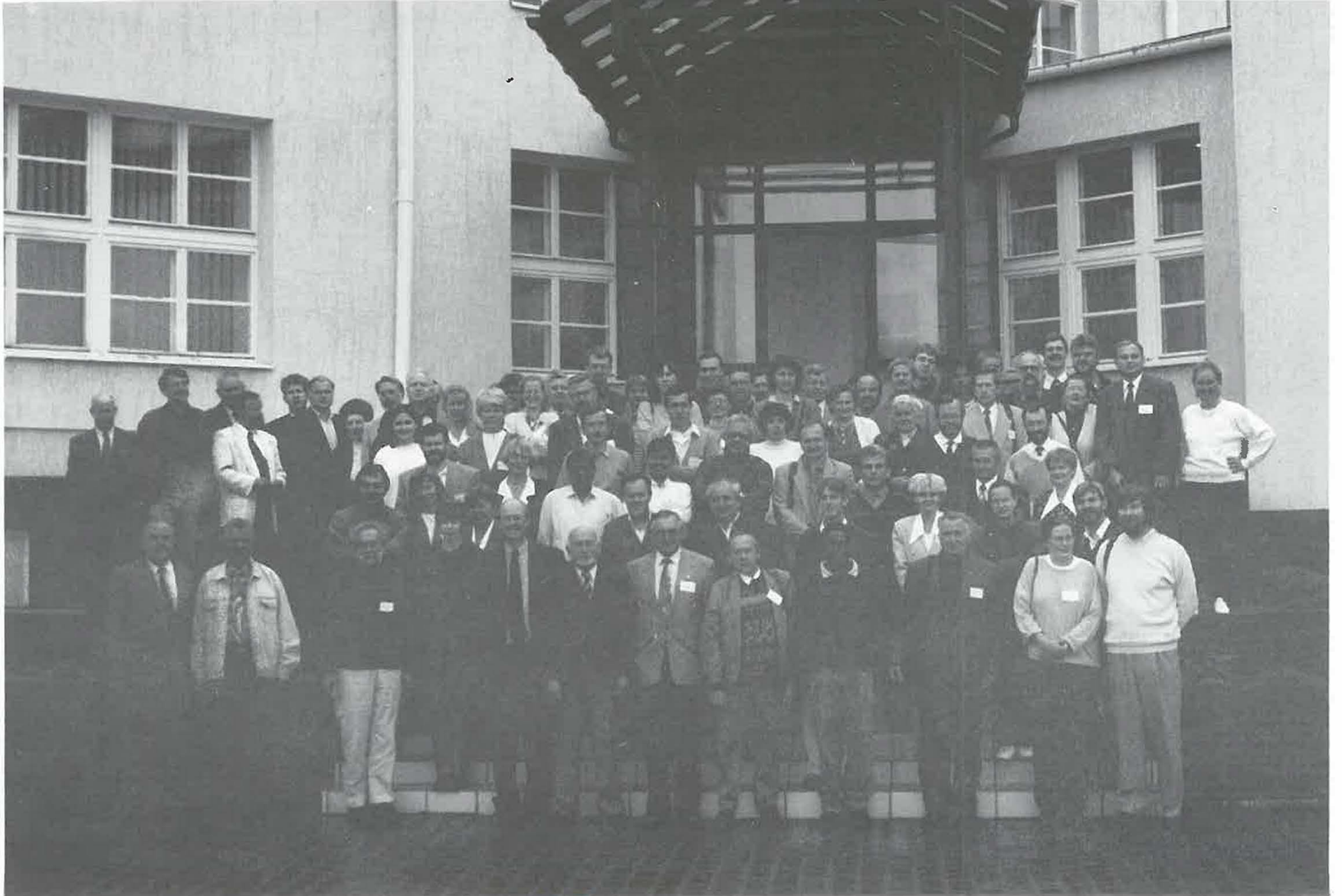
Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Régionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat:
INRA Station de Zoologie
Domaine Saint-Paul
Site Agroparc
84914 AVIGNON Cedex 9
France

ISBN 92-9067-083-5

1. M.P. Lesovoj
2. M.G. Ciuhrii
3. J. Bresciani
4. P.H. Smits
5. J.J. Lipa
6. J. Huber
7. O.W. Smirnov
8. G.S. Ochiel
9. V.T. Goncharov
10. T. Steenberg
11. H. Hokkanen
12. S. Wolny
13. A. Łukowicz
14. J. Kowalska
15. J. Jarosz
16. R. Wojciechowska
17. G. Galani
18. D. Stephan
19. C. Santiago-Alvarez
20. D. Segal
21. D. Chudaś
22. I. Vänninen
23. A.F. Pedersen
24. E. Tarasco
25. M. Andermatt
26. B. Płonka
27. J.S. Miduturi
28. D. Sulistyanto
29. O. Triggiani
30. J. Piątkowski
31. B. Papierok
32. A.C. Chapple
33. M. Janasik
34. C. Tkaczuk
35. A. Sapieha
36. R.-U. Ehlers
37. R.P. Bateman
38. G. Zimmermann
39. R. Miętkiewski
40. A. Peters
41. F. Ranaivo
42. K. Bolckmans
43. J. Eilenberg
44. I.V. Issi
45. L.T. Voloshchuk
46. C. Bajan
47. J. Coosemans
48. E. Popowska-Nowak
49. M. Stefaniak
50. W.J. Ravensberg
51. R. van der Pas
52. K. Dromph
53. C. Griffin
54. L. Rovesti
55. L. Gerritsen
56. C. Nielsen ?
57. E. Vargas-Osuna
58. R. Wegensteiner
59. P. Fitters
60. H. Malinowski
61. K. Jung
62. C. Mätje
63. J. Ziemnicka
64. A. Neumann
65. J. Piątkowski
66. R.V. Borovik
67. V. Shevtsov
68. A. Sierpińska
69. N. Lappa
70. V.A. Pavlyushin
71. P. Koppert
72. A. Bednarek
73. W. Goszczyński
74. Z. Mracek
75. G.G. Hauptmann


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75



Introduction

This Bulletin contains the proceedings of the first combined meeting of the IOBC/WPRS and IOBC/EPRS Working Groups on Insect Pathogens and Insect Parasitic Nematodes. The meeting was organised and hosted by Professor Jerzy Lipa and his team of the Institute of Plant Protection Poznan, Poland. The meeting took place August 27 to September 1, 1995 and was attended by more than 100 scientists from 21 countries, equally divided between east and west.

At the meeting 75 papers or posters were presented on insect pathogenic bacteria, viruses, fungi, nematodes and protozoa. Special themes at the meeting were microbial control of forest pest and application technology.

On behalf of the Working Groups I would like to thank Jerzy Lipa and his staff for the excellent manner in which they organised the meeting at the very suitable new conference center in Poznan. The meeting was fruitful in the exchange of scientific findings and ideas. But even more important perhaps was the fact that it was the first time large numbers of researchers in the field of insect pathology from Eastern and Western Europe met in person and got in touch with each other. I hope and expect this will lead to much better contacts and exchange of information and ideas than in the past.

The next general meeting of the Working Group will be held in Copenhagen, Denmark, in August 1997. The theme of the meeting will be " the role of entomopathogens in sustainable agriculture". The next combined meeting with the EPRS Working group is planned for 1999 in St. Petersburg. I hope that many of you will again attend these working group meetings.

Peter Smits
Convener

LIST OF PARTICIPANTS

ABOL-ELA Said, Dr.
ORSTOM, Entomovirology Laboratory
P.O. 26 Giza
12211 Cairo / Egypt
Tel. 202-5702134 / Fax 202-703948

ANDERMATT Martin, Dr.
Andermatt BIOCONTROL AG
Underdorf
CH-6146 Grossdietwil / Switzerland
Tel. 063-592840 / Fax 063-592123

BAJAN Cecylia, Prof. Dr.
Institute of Ecology PAS
05-092 Dziekanów Leśny / Poland
Tel. 4822-7513046 / Fax 4822-513100

BAŁAZY Stanisław, Prof. Dr.
Research Centre for Agricultural
and Forest Environment
Bukowska 19
60-809 Poznań / Poland
Tel. 4861-475601 / Fax

BATEMAN Roy P., Dr.
International Institute of Biological Control
Silwood Park, Ascot
Berks SL5 7TA / UK
Tel. 01344-294383 / Fax 01344-875007

BEDNAREK Andrzej, Dr.
SGGW - Department of Zoology
Nowoursynowska 166
02-975 Warszawa / Poland
Tel. 022-423056 / Fax 022-421556

BOEMARE Noël, Dr.
INRA, Lab. de Pathologie Comparee UM II
34095 Montpellier Cedex 5 / France
Tel. 33-67143740 / Fax 33-67144679

BOGNAR Csaba
ELTE Budapest
Department of Genetics
H-1088 Budapest / Hungary
Tel. 36-1-2661296 / Fax 36-1-2662694

BOLCKMANS Karel
BIOBEST N.V.
Ilse Velden 18
B-2260 Westerlo / Belgium
Tel. 32-14-231801 / Fax 32-14-231831

BOROVIK Roman V., Dr.
Research Centre of Toxicology and Hygienic
Regulation of Biopreparation
P.O. Dashkovka, Lenin str. 102 A
Serpukhov district
142283 Moscow region / Russia
Tel. 725578 / Fax 725578

BRESCIANI Jose, Dr.
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-35282662 / Fax 45-35282670

CHAPPLE Andrew C., Dr.
Ecogen Europe Srl
3A Parco Tecnologico Agro-Alimentare dell'Umbria
06050 Todi, Frazione Pantalla (PG) / Italy
Tel. 39 758957236 / Fax 39 75888776

CIUHRII Mircea G., Prof. Dr.
Research Institute for Plant Protection
Bd. Ion Ionescu de la Brad, 8
71592 Bucuresti / Romania
Tel. 40-1-6335850 / Fax 40-1-6335361

COOSEMANS J., Dr.
Lab. Fytopathologie EN
Katholieke Univ. Leuven
Willem de Croylaan 42
B-3001 Heverlee - Leuven / Belgium
Tel. 3216322737 / Fax 3216322989

DAMGAARD Per H.
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-3528-2660 / Fax 45-3528-2670

DOMAGAŁA Tomasz, Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

DROMPH Karsten
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-35282672 / Fax 45-35282670

EHLERS Ralf-Udo, Dr.
Institut für Phytopathologie CAU-Kiel
Klausdorferstr. 28-36
2313 Raisdorf / Germany
Tel. 49-4307-7498 / Fax 49-4307-7499

EILENBERG Jorgen, Prof. Dr.
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-35282692 / Fax 45-35282670

FITTERS Paul
St. Patrick's College
Maynooth Co. Kildare / Ireland
Tel. 353-1-6285222 / Fax 353-1-7083845

FJELSTED PEDERSEN Anita
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 35282692 / Fax 35282670

FODOR Andras, Dr.
ELTE Budapest
Department of Genetics
H-1088 Budapest / Hungary
Tel. 36-1-2661296 / Fax 36-1-2662694

GALANI Gheorghe, Dr.
Research Institute for Plant Protection
Bd. Ion Ionescu de la Brad, 8
71592 Bucuresti / Romania
Tel. 40-1-6335850 / Fax 40-1-6335361

GERRITSEN Lonne
IPO-DLO
P.O.Box 9060
6700 GW Wageningen / Netherlands
Tel. 31-3174-76001 / Fax 31-3174-10113

GŁOWACKA Barbara, Prof. Dr.
Forest Research Institute
Bitwy Warszawskiej 1920 r. nr 3
00-973 Warszawa / Poland
Tel. 022-223201 / Fax 022-224935

GONCHAROV V. T., Dr.
All-Russian Institute of Biological Control
350039, Krasnodar p/o 39 / Russia

GRIFFIN Christine, Dr.
Biological Department
St. Patrick's College
Maynooth Co. Kildare / Ireland
Tel. 353-1-7083841 / Fax 353-1-7083845

HAUPTMANN Georg-Gerhart, Dr.
Hamburg University
Inst. of Applied Botany / Plant protection Div.
P.O. Box 302762
D-20309 Hamburg / Germany
Tel. 49-40-41232359 / Fax 49-40-41236593

HOKKANEN Heikki, Prof. Dr.
Department of Applied Zoology
University of Helsinki
Box 27
00014 Helsinki-Viikki C / Finland
Tel. 00358-0-7085371 / Fax 00358-0-7085463

HUBER Jürg, Prof. Dr.
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 49-6151-40720 / Fax 49-6151-40790

ISSI Irma V., Prof. Dr.
All-Russian Institute for Plant Protection
Podbelskogo 3
St. Petersburg-Pushkin, 189620 / Russia
Tel. 812-4768872 / Fax 812-4764388

JAROSZ Jan, Prof. Dr.
UMCS - Institute of Biology
Akademicka 19
20-033 Lublin / Poland
Tel. 4881-375931 / Fax 4881-375102

JUNG Kerstin
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 49-6151-40736/ Fax 49-6151-40790

KISS Zsuzsanna
ELTE Budapest
Department of Genetics
H-1088 Budapest / Hungary
Tel. 36-1-2661296 / Fax 36-1-2662694

KRASOMIL-OSTERFELD Karina, Dr.
ECOGEN - Bio Germany
Klausdorferstr. 28-36
24223 Raisdorf / Germany
Tel. 49-4307-7496 / Fax 49-4307-7495

LAPPA Nina V., Dr.
Institute of Plant Protection
Vasilkovskaya 33
252022 Kiev-22 / Ukraine
Tel. 38044-2631124 / Fax 38044-2632185

LESOVOJ Michail P., Prof. Dr.
Institute of Plant Protection
Vasilkovskaya 33
252022 Kiev-22 / Ukraine
Tel. 38044-2631124 / Fax 38044-2632185

LINDQUIST Richard K., Dr.
OARDC, Department of Entomology
1680 Madison Avenue
Wooster, OH 44691-4096 / USA
Tel. 216-2633736 / Fax 216-2633686

LIPA Jerzy J., Prof. Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-675051 / Fax 4861-676301

ŁUKOWICZ Anna
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

MACHOWICZ-STEFANIAK Zofia, Prof. Dr.
AR - Chair of Phytopathology
Leszczyńskiego 7
20-069 Lublin / Poland
Tel. 4881-23047

MAICHROWICZ Irena, Prof. Dr.
AR - Chair of Entomology
Slowackiego 17
70-454 Szczecin / Poland
Tel. 4891-76071 / Fax 4891-71962

MALINOWSKI Henryk, Prof. Dr.
Forest Research Institute
Bitwy Warszawskiej 1920 r. nr 3
00-973 Warszawa / Poland
Tel. 4822-223201 / Fax 4822-224935

MARTON Palma
ELTE Budapest
Department of Genetics
H-1088 Budapest / Hungary
Tel. 36-1-2661296 / Fax 36-1-2662694

MÄTJE Carmen
Institut für Phytopathologie CAU-Kiel
Klausdorferstr. 28-36
2313 Raisdorf / Germany
Tel. 49-4307-7498 / Fax 49-4307-7499

MICHALIK Joanna, Dr.
Institute of Biochemistry and Biophysics
Pawińskiego 5a
02-106 Warszawa / Poland
Tel. 659-70-72 / Fax 48-39-12-16-23

MIDUTURI John S.
Research Station for Nematology and Entomology
Brug. Van Gansberghelaan 96
B-9820 Merelbeke / Belgium
Tel. 32-9-2720275 / Fax 32-9-2720215

MIĘTKIEWSKI Ryszard, Prof. Dr.
WSRP - Chair of Plant Protection
Prusa 14
08-110 Siedlce / Poland
Tel. 4825-25295

NEUMANN Anja
Institut für Phytopathologie CAU-Kiel
Klausdorfer str. 28-36
2313 Raisdorf / Germany
Tel. 49-4307-7498 / Fax 49-4307-7499

NIELSEN Charlotte
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-35282672 / Fax 45-35282670

OCHIEL Gerard S.
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1890 Frederiksberg C / Denmark
Tel. 45-35282692 / Fax 45-35282670

O'NEILL Keith
Biology Department
St. Patrick's College
Maynooth Co. Kildare / Ireland
Tel. 353-1-6285222 / Fax 353-1-7083845

PAPIEROK Bernard, Dr.
Institut Pasteur
25, rue du Dr Roux
75724 Paris Cedex 15 / France
Tel. 33-1-45688226 / Fax 33-1-40613471

PAVLYUSHIN V.A., Dr.
All-Russian Institute for Plant Protection
Podbelskogo 3
St. Petersburg-Pushkin, 189620 / Russia
Tel. 812-4768872 / Fax 812-4764388

PETERS Arne, Dr.
Ecogen Europe Srl.
3A Parco Tecnologico Agro-Alimentare dell'Umbria
06050 Todi, Frazione Pantalla (PG) / Italy
Tel. 3975-8957236 / Fax 3975-888776

PIĄTKOWSKI Janusz, Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

PIĄTKOWSKI Jerzy
UWr - Institute of Microbiology
Przybyszewskiego 63
51-148 Wrocław / Poland
Tel. 4871-252151 / Fax 4871-252151

POPOWSKA-NOWAK Elżbieta
Institute of Ecology PAS
05-092 Dziekanów Leśny / Poland
Tel. 4822-7513046 / Fax 4822-513100

PRILEPSKAJA Nadezhda, Dr.
IOBC/EPRS Secretariat
Sadovaya-Spasskaya 18
107807 Moscow b-78/ Russia

PRISHCHEPA Ludmila, Dr.
Belorussian Institute of Plant Protection
P/O Priluki
223011 Minsk / Belarus
Tel. 70172-772150 / Fax 70172-992338

RANAIVO Fidy
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 49-6151-40737 / Fax 49-6151-40790

RAVENSBERG Willem J., Dr.
Koppert B.V.
Veilingweg 17,
Postbus 155
2650 AD Berkel en Rodenrijs / Netherlands
Tel. 31-1891-40444 / Fax 31-1891-15203

ROPEK Dariusz
AR - Chair of Plant Protection
Al. 29 Listopada 48
31-425 Kraków / Poland
Tel. 4812-119144 / Fax 4812-336245

ROVESTI Luciano, Dr.
Centro di Studio del Fitofarmaci
via Filippo Re, 8
40126 Bologna / Italy
Tel. 3951-351359 / Fax 3951-351364

SADOMOW Edward
IOBC/EPRS Secretariat
Sadovaya-Spasskaya 18
107807 Moscow b-78/ Russia

SANTIAGO-ALVAREZ Candido, Prof. Dr.
E.T.S.I.A.M. Universidad de Cordoba
Apartado 3048
140808 Cordoba / Spain
Tel. 57-218475 / Fax 57-298343

SAPIEHA Anna, Dr.
WSRP - Chair of Plant Protection
Prusa 14
08-110 Siedlce / Poland
Tel. 4825-25295

SEGAL Daniel, Dr.
Dept. of Molecular Microbiology
and Biotechnology
Tel-Aviv University
69978 Tel-Aviv / Israel
Tel. 972-3-6409835 / Fax 972-3-6409407

SHEVTSOV Valeri, Dr.
Biotechnological Innovation Centre
P.O. Dashkovka
102A Lenin str., Serpukhov Dis.
142283 Moscow Region / Russia
Tel. 095-1168980 / Fax 095-1168980

SIERPIŃSKA Alicja
Forest Research Institute
Bitwy Warszawskiej 1920 r. nr 3
00-973 Warszawa / Poland
Tel. 4822-223201 / Fax 4822-224935

SKRZYPEK Henryk, Dr.
KUL - Department of Environmental Biology
Norwida 4
20-950 Lublin / Poland
Tel. 4881-33784

SMIRNOV O.W., Dr.
Research Institute for Agricultural Microbiology
Podbelskogo 3
St. Petersburg, Pushkin, 189620 / Russia

SMITS Peter H., Dr.
IPO-DLO
P.O. Box 9060
6700 GW Wageningen / Netherlands
Tel. 31-3174-76103 / Fax 31-3174-10113

SOSNOWSKA Danuta, Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

STEENBERG Tove
Danish Pest Infestation Laboratory
Skovbrynet 14
2800 Lyngby / Denmark
Tel. 45-45878055 / Fax 45-45931155

STEFANIAK Malgorzata
UMCS - Institute of Biology
Akademicka 19
20-033 Lublin / Poland
Tel. 4881-375089 / Fax 4881-33669, 375102

STEPHAN Dietrich
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 06151-40738 / Fax 06151-40790

SULISTYANTO Didik
Institut für Phytopathologie CAU-Kiel
Klausdorferstr. 28-36
2313 Raisdorf / Germany
Tel. 49-4307-7498 / Fax 49-4307-7499

SZALLAS Emilia
ELTE Budapest
Department of Genetics
H-1088 Budapest / Hungary
Tel. 36-1-2661296 / Fax 36-1-2662694

TARASCO Eustachio, Dr.
Institute of Agricultural Entomology
University of Bari
via Amendola 165/A
70126 Bari / Italy
Tel. 080-5442879 / Fax 080-5442876

THOMSEN Lene
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-3528-2660 / Fax 45-3528-2670

TKACZUK Cezary, Dr.
WSRP - Chair of Plant Protection
Prusa 14
08-110 Siedlce / Poland
Tel. 4825-25295

TOMALAK Marek, Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

TRIGGIANI Oreste, Prof. Dr.
Institute of Agricultural Entomology
University of Bari
via Amendola 165/A
70126 Bari / Italy
Tel. 080-5442878 / Fax 080-5442876

VAN DER PAS R.
Koppert B.V.
Veilingweg 17,
P.O.Box 155
2650 AD Berkel en Rodenrijs / Netherlands
Tel. 31-1891-40444 / Fax 31-1891-15203

VÄNNINEN Irene, Dr.
Agricultural Research Centre of Finland
Institute of Plant Protection
31600 Jokioinen / Finland
Tel. 358-16-41881 / Fax 358-16-4188584

VARGAS-OSUNA Enrique, Dr.
E.T.S.I.A.M., Universidad de Cordoba
Apartado 3048
14080 Cordoba / Spain
Tel. 57-218476 / Fax 57-298343

VOLOSHCHUK Leonid T., Dr.
Institute for Biological Control
Dacia Bd. 58
Kishinev, 277060 / Moldova
Tel. 37-32-570485 / Fax 37-32-579641

WEGENSTEINER Rudolf, Dr.
Institute of Forest Entomology
University of Agriculture
Hasenauerstraße 38
1190 Vienna / Austria
Tel. 43-1-3195539-30 / Fax 43-1-3195539-97

WOLNY Stefan, Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

ZABŻA Andrzej, Prof. Dr.
WrTU - Institute of Organic Chemistry,
Biochemistry and Biotechnology
Wybrzeże Wyspiańskiego 27
50-370 Wrocław / Poland
Tel.4871-203446 / Fax 4871-203503

ZIEMNICKA Jadwiga, Dr.
Institute of Plant Protection
Miczurina 20
60-318 Poznań / Poland
Tel. 4861-679021 / Fax 4861-676301

ZIMMERMANN Gisbert, Dr.
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 06151-40728 / Fax 06151-40790

LIST OF RELATED PERSONS

AMARAL Joao J.S., Dr.
Serv.de Desenvolvimento Agrario da Terceira
Vinha Brava
9700 Angra do Heroismo / Portugal
Tel. 351-9523003 / Fax 351-95-23006

ANAGNOU-VERONIKI Maria, Dr.
NAGREF and Benaki Phytopathological Institute
8, Delta str.
14561 Kiphisia / Greece
Tel. 30-1-8077498 / Fax 30-1-8077506

ANDREI Ana Maria
Research Institute for Plant Protection
Bd. Ion Ionescu Dela Brad 8
71592 Bucharest / Romania
Tel. 40-1-6335850 / Fax 40-1-6335361

ARZONE Alessandra, Prof. Dr.
Dipartimento di Entomologia e Zoologia
Applicate All'Ambienie Carlo Vidano
Via Pietro Giuria 15
10126 Torino / Italy
Tel. 39-11-6505644 / Fax 39-11-6687016

BATHON Horst, Dr.
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 06151-40725 / Fax 06151-40790

BATUEV Sergey L.
Institute of Zoology
480032, Akademgorodok, Almaty / Kazakhstan
Tel. 481732 / Fax 07-3272-481958

BEKTURGANOV B.
Institute of Zoology
480032, Akademgorodok, Almaty / Kazakhstan
Tel. 481732 / Fax 07-3272-481958

BRANDL Franz, Dr.
Ciba Agro Germany
Liebigstr. 51-53
60323 Frankfurt / Germany
Tel. 069-7155232 / Fax 069-7155300

BEERLING E.
University of Amsterdam
Kruislaan 320
1098 SM Amsterdam / Netherlands
Tel. 31-205257745 / Fax 31-205257754

BRIXEY Julia M., Dr.
Forestry Authority, NRS
Roslin, Midlothian EH25 9SY / Great Britain
Tel. 44-1314452176 / Fax 44-1314455124

BUJAK Justyna
Institute of Organic Chemistry
Biochemistry and Biotechnology, WrTU
Wybrzeże Wyspiańskiego 27
50-370 Wrocław / Poland
Tel. 4871-202455 / Fax 4871-223664

CAROLI Luigi, Dr.
Ecogen Europe Srl
Frazione Pantalla
I-06050 Pantalla (PG) / Italy
Tel. 39-758957236 / Fax 39-75888776

CHERKASHIN Aleksandr N.
Institute of Zoology
480032, Akademgorodok, Almaty / Kazakhstan
Tel. 481732 / Fax 07-3272-481958

CHERRY Andy, Dr.
NRI
Central Ave.
Chatham Maritime, Kent, ME4 4TB / UK
Tel. 634-883330 / Fax 634-880066/77

CORY Jenny S., Dr.
NERC Institute of Virology
Mansfield Road
Oxford, OXI 3SR / UK
Tel. 1865-512361 / Fax 1865-59962

CRAVANZOLA Federica, Dr.
Univ. di Torino, DI. VA. P.R.A.
- Microbiologia e Industrie agrarie
via P. Giuria, 15
Torino / Italy
Tel. 39-11-6687960 / Fax 39-11-6502139

CROITORU Nichita I., Dr.
Universitatea agrara de Stat din Moldova
Mircesti, 44
Chisinau, 49 / Moldova
Tel. 432582

DABERT Mirosława
Zakład Biochemii Biopolimerów, UAM
Międzychodzka 5
60-371 Poznań / Polska
Tel. 4861-615596

DAOUST Richard A., Dr.
Ecogen Europe Srl
Frazione Pantalla di Todi
06050 Todi-Perugia / Italy
Tel. 39-75-8957236 / Fax 39-75-888776

DUBITSKII Anatoly M.
Institute of Zoology
480032, Akademgorodok, Almaty / Kazakhstan
Tel. 481732 / Fax 07-3272-481958

ELLAR David J., Dr.
Department of Biochemistry
University of Cambridge
Tennis Court Road
Cambridge CB2 1QW / UK
Tel. 44-1223-333651 / Fax 44-1223-333345

FEDIERE Gilles, Dr.
ORSTOM, Entomovirology Laboratory
P.O. 26, Giza
12211 Cairo / Egypt
Tel. 202-5702134 / Fax 202-3609286

FIGUEIREDO Elisabete, Dr.
Instituto Superior de Agronomia - SAPI
Tapada da Ajuda
1399 Lisboa Codex / Portugal
Tel. 351-1-3638161 / Fax 351-1-3635031

GARCIA DEL PINO Fernando, Dr.
Dep. Biologia Animal, Facultad de Ciencias
Universidad Autonoma Barcelona
08193 Bellaterra-Barcelona / Spain
Tel. 3-5811841 / 3-5811321

GERBER Karin, Dr.
Federal Agency & Res. Centre for Agriculture
Trunnerstr. 5
A-1020 Vienna / Austria
Tel. 43-1-21113254 / Fax 43-1-2160825

GILLESPIE A.T., Dr.
Chr. Hansens Biosystems A/S
Bose Alle 10-12
DK-2970 Horsholm / Denmark
Tel. 75-766666 / Fax 75-766066

GOLEMANSKY Vassil, Prof. Dr.
Institute of Zoology
Bld. "Tzar Osvoboditel", 1
1000 Sofia / Bulgaria
Tel. 359-2-884708 / Fax 359-2-882897

GREB-MARKIEWICZ B.
Inst. Org. Chem., Biochem. and Biotechn.,
Technical University
Wybrzeże Wyspiańskiego 27
50-370 Wroclaw / Polska
Tel. 4871-203446 / Fax 4871-202415

GRUNDER Jürg, Dr.
Swiss Federal Research Station
CH-8820 Wadenswil / Switzerland
Tel. 41-1-7836336 / Fax 41-1-7806341

GUILLON Michel, Dr.
N.P.P.
BP 80, Route Diartix
64150 Nogueres / France
Tel. 33-59609292 / Fax 33-59609219

GÜRLICH Gunhild
BBA - Institute for Biological Control
Heinrichstr. 243
D-64287 Darmstadt / Germany
Tel. 6151-40732 / Fax 6151-40790

HASS Birgit
Biology Dept., St. Patrick's College
Maynooth
Co. Kildare / Ireland
Tel. 353-1-6285222 / Fax 353-1-7083845

HYSLOP D.P.
AgrEvo Environmental Health Ltd.
Berkhamsted, Herts HP4 2DY / UK
tel. 01442-863333 / Fax 01442-861307

JAWORSKA Magdalena, Doc. dr
AR - Chair of Plant Protection
Al. 29 Listopada 48
31-425 Kraków / Poland
Tel. 4812-119144 / Fax 4812-336245

KAMIONEK Marta, Dr. habil.
SGGW, Katedra Zoologii
Nowoursynowska 166
02-975 Warszawa / Polska
Tel. 4822-439081

KANDYBIN N.V., Prof. Dr.
Research Institute for Agricultural Microbiology
Podbelskogo 3
St. Petersburg, Pushkin, 189620 / Russia

KELLER Brigitte, Dr.
BBA - Institute for Biological Control
Heinrichstr. 243
64287 Darmstadt / Germany
Tel. 49-6151-40729 / Fax 49-6151-40790

KELLER Siegfried, Dr.
Federal Research Station for Agronomy
Reckenholzstrasse 191/211
CH-8046 Zurich / Switzerland
Tel. 01-3777211 / Fax 01-3777201

KILION Michael, Dr.
Bayer AG, Geschäftsbereich Pflanzenschutz
Pflanzenschutzzentrum Monheim
D-51368 Leverkusen / Germany
Tel. 02173-383210 / Fax 02173-383150

KLEESPIES R.G., Dr.
BBA - Institute for Biological Control
Heinrichstr. 243
D-64287 Darmstadt / Germany
Tel. 6151-4070 / Fax 6151-40790

KUZMANOVA I., Prof. Dr.
Kafedra Mikrobiologii Selkhozinstituta
Mendeleev 12
Plovdiv / Bulgaria

LACEY Lawrence A., Dr.
USDA, European Biological Control Lab.
BP 4168 Agropolis
Montpellier 34092 / France
Tel. 3367526844 / Fax 3367619993

MARINI Giulio
3 A c/o Ecogen Bio Germany
Klausdorfer str. 28-36
24223 Raisdorf / Germany
Tel. 49-4307-7496 / Fax 49-4307-7496

MOORE D., Dr.
IIBC
Silwood Park
Ascot, Berks SL5 7TA / UK
Tel. 01344-872999 / Fax 01344-875007

MUSIAŁ Wojciech
Zakład Biochemii Biopolimerów, UAM
Międzychodzka 5
60-371 Poznań / Polska
Tel. 4861-615596

NOVIKOVA I.I. Dr.
All-Russian Institute for Plant Protection
Podbelski sh., 3
St. Petersburg-Pushkin, 189620 / Russia
Tel. 4768693 / Fax 7812-4764388

PARAISO Armand, Dr.
IITA
BP: 12625
Niamey / Niger
Tel. 732053 / Fax 732237

PASQUALINI Edison, Dr.
Inst. of Entomology - University of Bologna
Via Filippo RE, 6
40126 Bologna / Italy
Tel. 39-51-251052 / Fax 39-51-351559

PEDERSEN Amelie
National Chemicals Inspectorate
P.O.Box 1384
S-171 27 Solna / Sweden
Tel. 46-8-7305700 / Fax 46-8-7357698

PEZOWICZ Elżbieta, Dr.
SGGW, Katedra Zoologii
Nowoursynowska 166
02-766 Warszawa / Polska
Tel. 4822-439081

PIATTI Piergiovanni, Dr.
Univ. di Torino
Dip. Microbiologia e Industrie agrarie
via P. Giuria, 15
Torino / Italy
Tel. 39-11-6687960 / Fax 39-11-6502139

PILARSKA Daniela, Dr.
Institute of Zoology BAS
Bld. "Tzar Osvoboditel", Nr. 1
1000 Sofia / Bulgaria
Tel. 359-2-885115 / Fax 359-2-882897

POPUSHOY I.S., Prof. Dr.
Institute for Biological Control
Dacias Bd. 88
Kishinev, 277060 / Moldova

PRIOR C., Dr.
IIBC
Silwood Park
Ascot, Berks SL5 7TA / UK
Tel. 01344-872999 / Fax 01344-875007

PROTA Romolo, Prof. Dr.
CNR/IRCBA c/o Istituto Ent. Agrar. Universita
Via E. De Nicola
Sassari / Italy
Tel. 39-79-229246 / Fax 39-79-229329

RAGNI Adriano
Ecogen Europe Srl
Frazione Pantalla
I-06050 Pantalla-Perugia / Italy
Tel. 39-75-8957236 / Fax 39-75-888776

RASPOPOV Andrey P.
Federal Forest Service of Russia
59/19, Pyatnitskogo str.
Moscow, 113184 / Russia
Tel. 095-2308682 / Fax 095-2308682

RICCI Manuele, Dr.
Ecogen Europe Srl
Frazione Pantalla
06050 Todi (PG) / Italy
Tel. 39-75-8957236 / Fax 39-75-888776

RICHARDSON Paul, Dr.
Entomology Department HRI
Worthing Road
Littlehampton BN17 6CP / UK
Tel. 0903-716123 / Fax 0903-726780

SAMERSOV V.F., Prof. Dr.
Belorussian Institute of Plant Protection
P/O Priluki
223011 Mińsk / Bielorus
Tel. 70172-772150 / Fax 70172-992338

SAMOJLOV Y.K., Dr.
ITI Biotechnique
Bolshaya Arnautskaya 10
270012 Odessa / Ukraine

SCHIROCKI Anke, Dr.
Univ. of Reading, Dep. of Agriculture
Earley Gate
Reading RG6 2AT / England
Tel. 0734-875123 / Fax 0734-352421

SEMENCHENKO Galina V.
Institute of Zoology
480032, Akademgorodok, Almaty / Kazakhstan
Tel. 481732 / Fax 07-3272-481958

SERMANN Helga, Dr.
Humboldt-Univ., FG Angew. Entomologie
Dorfstr. 9
D-13051 Berlin-Malchow / Germany
Tel. 030-9650489 / Fax 030-9650235

SKIRKEVICIUS Algirdas, Dr.
Institute of Ecology
Akademijos 2
2600 Vilnius / Lithuania
Tel. 3702-359274 / Fax 3702-359257

SKOVMAND Ole, Dr.
ORSTOM Montpellier, LIN
911, Av. Agropolis
B.P. 5045
34032 Montpellier Cedex 1 / France
Tel. 33-67041924 / Fax 33-67542044

SOKOLOV M.S., Prof. Dr.
All-Russian Institute of Biological Control
350039, Krasnodar p/o 39 / Russia

STEVENS Peter, Dr.
OSi Specialties, Inc.
777 Old Saw Mill River Road
Silicones Building
Tarrytown, NY 10591-6728 / USA
Fax 1-914-7893102

STROHHÄCKER Joachim, Dr.
Ecogen - Bio Germany
Klausdorfer str. 28-36
24223 Raisdorf / Germany
Tel. 49-4307-7496 / Fax 49-4307-7495

SZEWCZYK Dorota
BBA - Institute for Biological Control
Heinrichstr. 243
D-64287 Darmstadt / Germany
Tel. 6151-40755 / Fax 6151-40790

TCHUBIANISHVILI T., Prof. Dr.
Georgian Institute of Plant Protection
Tchavtchavadze 82
380062 Tbilisi / Georgia

THIERY Isabella, Dr.
Institut Pasteur
25 Rue du Dr Roux
75724 Paris Cedex 15 / France
Tel. 33-1-40613183 / Fax 33-1-40613044

VAN TOL R.W.
Research Station for Nursery Stock
P.O. Box 118
2770 A.C. Boskoop / Netherlands
Tel. 01727-19797 / Fax 01727-19717

VELICHKOVA-KOZHUKHAROVA M., Dr.
Institute of Plant Protection
Sofia-Kostinbrod / Bulgaria

VESTERGAARD Susanne
KVL - Dept. of Ecology and Molecular Biology
13, Bülowsvej
1870 Frederiksberg C / Denmark
Tel. 45-35282684 / Fax 45-35282670

VLAK Just M., Dr.
Dept.of Virology, Agric. University
P.O. Box 8045
6700 EM Wageningen / Netherlands
Tel. 31-8370-83090 / Fax 31-8370-84820

VORONINA Elina G., Prof. Dr.
All-Russian Institute for Plant Protection
Podbelskogo 3
St. Petersburg, Pushkin, 189620 / Russia
Tel. 812-4768872 / Fax 812-4764388

VRIESEN Silvia
BBA - Institute for Biological Control
Heinrichstr. 243
D-64287 Darmstadt / Germany
Tel. 6151-40754 / Fax 6151-40790

WITASEK Peter
Pa. Kornels Bio
Dietrichsteinerstr. 14
A-9560 Feldkirchen / Austria
Tel. 43-4276-3230 / Fax 43-4276-268818

ZUBER Markus, Dr.
Andermatt BIOCONTROL AG
Wasserfluestr. 25
5024 Kuttigen / Switzerland
Tel. 063-592840 / Fax 063-592123

TABLE OF CONTENTS

3. Entomopathogenic Nematodes & their Symbiotic Bacteria

4. Entomopathogenic Fungi

1. General Insect Pathology

INSECT PATHOLOGY AND MICROBIAL CONTROL IN THE EPRS REGION AND IN POLAND

Jerzy J. Lipa
Department of Biological Control & Quarantine, Institute of Plant Protection,
Miczurina 20, 60-318 Poznan, Poland

Abstract. A concise information is provided on activities of the IOBC/EPRS Standing Commission on Microbiological Means for Plant Protection established in 1977 including meetings, proceedings and articles published in the *Information Bulletin EPS IOBC*. In addition, an information is given on other microbial control and on biopesticide production cooperative programs between countries of Central and Eastern Europe. In two Annexes the lists of pertinent publications are given.

INTRODUCTION

There is a tremendous number of research publications, conference proceedings, books and reviews concerning insect pathogens and their use in microbial pest control in the area covered by the IOBC East Palearctic Regional Section activity . Unfortunately, this interesting and important literature is almost unknown judging by the low citation index in the western literature. The reason for this neglect is quite simple. A great majority of these publications is written in Slavic languages and published in journals with local and very limited circulation. Although in many cases an English summary is provided but it is so short and enigmatic that in fact it does not provide any factual information. Due to above reasons many important findings and interesting ideas originating in the EPRS region remain unknown to specialists acting in the WPRS. A striking example of this situation is that the "*Information Bulletin EPS IOBC*" publishing articles in Russian is unknown to WPRS colleagues. On the other hand the "*WPRS Bulletin/Bulletin SROP*" with conference papers written mostly in English is quite well known to specialists active in the EPRS.

In the past I have made several attempts to inform insect pathologists and microbial control specialists of the WPRS region about various activities and achievements in the research on invertebrate pathology and microbial control of pests in the area covered by the IOBC/East Paleartic Regional Section (Lipa, 1973, 1980a,b, 1985, 1989, 1990a,b, 1991).

In this review I provide additional information on three large cooperative programmes concerning basic research and biotechnology goals existing during 1972-1991 as well as on IOBC/EPRS activivties initiated in 1977 and continued until the present time.

COMECON COOPERATION IN SCIENCE AND TECHNOLOGY (1972-1991)

In 1972 a Council of Mutual Economic Cooperation (COMECON) established several scientific and technlogical priorities. Among them was one concerning plant protection under the code KNTS-12/III.1 "Elaboration of the systems of pesticide application, biological means of plant protection and a complex study of their influence upon the environment". As a Coordinating Centre for this programme the Institute of Plant Protection in Poznan (Poland) has been selected (Lipa, 1980b). Among thirteen detailed projects was 1.2.3. "Studies of entomopathogenic microorganisms and development of technologies of their production and

application" in which collaborated the following countries: Bulgaria, Czechoslovakia, German Democratic Republic, Hungary, Poland, Romania and Soviet Union.

Within this programme the following five symposiums were organized:

- „Microbial Preparations for Plant Protection" (March 23-28, 1981, Moscow, USSR);
- „Recent Advances in Insect Pathology and Microbial Control" (April 11-17, 1983, Rytro, Poland);
- „Entomopathogenic Microrganisms and Their Diagnostic" (September 2-6, 1985, Kraimorie, Bulgaria);
- „Biotechnology of Entomopathogenic Fungus Preparations" (October 19-23, 1987, Bucharest, Romania);
- „Effectiveness of Microbial Means of Plant Protection" (May 15-18, 1989, Tiraspol, Ukraine);
- „II Symposium of COMECON Member Countries for Microbial Pesticides" (October 15-19, 1990, Protivno, USSR) (Anonymous, 1990).

Although several interesting papers have been presented at these meeetings no proceedings have been published, except the last. Nevertheless, some of the presented papers were published elsewhere.

BIOTECHNOLOGY COOPERATIVE PROGRAMME (1986-1991)

In 1986 an "Intergovernmental Agreement on Scientific-Technical Cooperation on Accelerated Development and Application of Biotechnology in Agriculture" has been signed by Bulgaria, Czechoslovakia, German Democratic Republic, Hungary, Cuba, Mongolia, Poland, Vietnam and the USSR. A task 5.1.2.1 "Elaboration of new microbial plant protection means including fungus, virus, bacterial and antibiotics" have been formulated. At first the Institute of Biotechnology for Microbial Plant Protection Means and Fertilizers in Moscow has been selected as the International Scientific Coordinating Centre but later this duty has been assigned to the All-Union Institute of Apied Microbiology in Obolensk. Technological and industrial aspects have been coordinated by scientific-industrial complexes NPO Biotechnologia in Moscow and NPO Biosintez in Obolensk, all belonging to the USSR Ministry of Medical and Biological Industry.

Each cooperating country established a national programme in biotechnology and in case of Poland the Institute of Plant Protection in Poznan has played the key-role in its realization.

The following tasks have been selected as main goals:

- optimizing prodution technology and formulations of *Bacillus thuringiensis* bioinsecticides;
- industrial technology of production of virus insecticides for control of *Lymantria, Mamestra, Agrotis, Malacosoma* and *Carpocapsa*;
- one-phase technology of mass growing of fungi *Beauveria, Verticillium, Aschersonia, Paecilomyces* and *Metarhizium* for production of mycoinsecticides Boverin, Boverol, Mikoafidin, Entomoftorin-T, Verticillin.

The cooperating teams had several management meetings devoted to: strain selection (August 5-8, 1986, Moscow, USSR; December 17-20, 1986, Poznan, Poland); mycoinsecticides (March 30 - April 3, 1987, Moscow, USSR), general aspects (November 23-27, 1987, Poznan, Poland; February 2-4, 1988, Leningrad, USSR), bacterial insecticides (December 12-17, 1988, Jabłonna, Poland); "mikovirin project" (October 3-4, 1989 Serpukhov, USSR).

In Poland the National Programme on Biotechnology helped to establish domestic production of two *B. thuringiensis* products (Bacillan and Thuridan) as well as Albak - plant

bacterial product (mixture of garlic and *B. thuringiensis*). However, attempts to establish production of mycoinsecticides based on *Beauveria bassiana* and *Verticillium lecanii* have failed.

IOBC/EPRS COOPERATION AND ACTIVITIES

The first attempts to organize the international organization of biological control on the basis of CILB (Commission International de Lutte Biologique) by joint action of specialists from Western and Eastern Europe go back to the time of the First International Conference on Insect Pathology and Biological Control in Prague August 13-18, 1958 (Anonymous, 1958a,b; Franz, 1958; Simmonds, 1958). However, this idea has materialized only in 1977 and the East Palearctic Regional Section of the IOBC has been organized. Among six permanent commissions there is the Standing Commission on Microbiological Means of Plant Protection which organized the following symposiums:

- „Principles of Registration and Evaluation of Entomopathogens" (September 14, 1978, Prague, Czechoslovakia);
- „Technical Characteristics of Microbial Preparations" (May 10-15, 1981, Prague , Czechoslovakia);
- „Insect Viruses and Perspectives of their Practical Use for Protection of Plants against Pests in Member Countries EPRS/IOBC" (November 19-21, 1980, Moskva, USSR) (Anonymous, 1981);
- „Ecological Grounds and Principles of Use of Biopesticides" (October 3-8, 1983, Sofia, Bulgaria);
- „Microbial Control of the Colorado Potato Beetle within Integrated System of Potato Protection" (October 28-November 1, 1985, Kiev, USSR/Ukraine);
- „Persistence and Effectiveness of Entomopathogens in Plant Protection" (April 6-11, 1987, Poznan, Poland) (Lipa, 1987);
- „Biotechnological Aspects of Microbial Control" (October 16-20, 1989, Hodmezevsharhei, Hungary.

INSECT PATHOLOGY AND MICROBIAL CONTROL IN POLAND

Detailed history and data on important contribution of Polish scientists e.g. Dzierżon, Cienkowski, Danysz or Wize to insect pathology and microbial history is given elsewhere together with the extensive bibliography (Anonymous, 1964; Lipa, 1963, 1974). Here I only want to emphasize the exceptionally important and revolutionary contributions of Siemaszko (1937) to the knowledge of *Beauveria* genus and of Nowakowski (1877, 1883) and Batko (1964a,b) to the knowledge and taxonomy of Entomophthorales (Bałazy, 1993).

REFERENCES

ANONYMOUS, 1958a. Transactions of the First International Conference of Insect Pathology and Biological Control. August 13 - 18, 1958. Prague (Czechoslovakia). Prague, 653 pp.

ANONYMOUS, 1958b. Resolutions of the 1st International Conference for Insect Pathology and Biological Control pp. 619-625. Transactions of the First International Conference of Insect Pathology and Biological Control. August 13 - 18, 1958. Prague (Czechoslovakia). Prague, 653 pp.

ANONYMOUS, 1964. Stan Badań nad Organizmami Pożytecznymi z Punktu Widzenia Potrzeb Ochrony Roślin w Polsce [State of Studies of Beneficial Organisms Useful for Plant

Protection in Poland]. Zeszyty Problemowe Postępów Nauk Rolniczych (Warszawa), No **45**, 222 pp.

ANONYMOUS, 1981. Virusy Nasekomykh i Perspektivy ikh Prakticheskogo Ispolzovaniya v Zashchite Rastenii ot Vrediteley v Stranakh-Chlenakh VPS/MOBB[Insect Viruses and Perspectives of their Practical Use for Protection of Plants against Pests in Member Countries EPRS/IOBC]. Symposium Proceedings 19-21.XI.1980. Moskva, 144 pp.

ANONYMOUS, 1990. II Simpozium Stran Chlenov SEV po Mikrobnym Pestitsidam. 15-19.X.1990, Protivno, SSSR. Tezisy Dokladov [II Symposium of COMECON Member Countries for Microbial Pesticides. 15-19 October 1990. Protivno, USSR. Abstracts of Papers]. Moskva, 202 pp.

BAŁAZY, S. 1993. Flora of Poland. Fungi. Vol. XXIV. Entomophthorales. Instytut Botaniki PAN, Kraków 356 pp.

BATKO, A. 1964a. Remarks on the genus *Entomophthora* Fresenius 1856 non Nowakowski 1883 (Phycomycetes: Entomophthoraceae). Bull. Acad. Pol. Sci., Ser. Sci Biol., **12(9)**: 319-321.

BATKO, A. 1964b. On the new genera: *Zoophthora* gen. nov., *Triplosporium* (Thaxter) gen.nov. and *Entomophaga* gen.nov. (Phycomycetes: Entomophthoraceae). Bull. Acad. Pol. Sci., Ser. Sci Biol., **12**: 323-326.

FRANZ, J. 1958. Die Internationale Kommission für biologische Schädlingsbekämpfung (C.I.L.B.) pp. 605-609. In „Transactions of the First International Conference of Insect Pathology and Biological Control". August 13 - 18, 1958. Prague (Czechoslovakia). Prague, 653 pp.

LIPA, J.J. 1963. Polish analytical bibliography of insect pathology. Part I. Diseases and microbial control of noxious insects. Prace Nauk. Inst. Ochr. Roślin **5(1)**: 3-101.

LIPA, J.J. 1973. Monitoring of the possible hazards of biological control agents during their operational use. World Health Organization Conference on the Safety of Biological Agents for Arthropod Control. Atlanta (USA) 16-19 April 1973, pp. 1-12.

LIPA, J.J. 1974. An Outline of Insect Pathology. USDA/NSF. Warsaw, 269 pp.

LIPA, J.J. 1980a. Progress in Microbial Control (1975-1980) World Activities. A. Eastern Europe. pp. 9-10. In "Proceedings of Workshop on Insect Pest Management with Microbial Agents: Recent Achievements, Deficiencies, and Innovations May 12-15, 1980". Boyce Thompson Institute, Cornell University, Ithaca, New York, 71 pp.

LIPA, J.J. 1980b. Main trends of research in biological control of plant pests in countries of Council of Mutual Economic Cooperations. Mat. XX Sesji Nauk. Inst. Ochr. Roślin pp. 315-324.

LIPA, J.J. 1985. History of biological control in protected crops: Eastern Europe. In „Biological Pest Control: The Glasshouse Experience" (N.W. Hussey & N. Scopoes, Eds.) Blandford Press, Poole-Dorset, pp. 232.

LIPA, J.J. (Ed.). 1987. Persistentnost i Effektivnost Insektitsidnykh Mikroorganizmov v Biotsenozakh. Materialy Simpoziuma VPS-MOBB [Persitence and Effectiveness of Insecticidal Microorganisms in the Biocenoses. Proceedings of a EPRS-IOBC Symposium]. Poznan 11-16.IV.1987. IOR, Poznan, 150 pp.

LIPA, J.J. 1989. Insect pathology and microbial control in Eastern Europe (EPRS-IOBC). Abstracts: "Second European Meeting on Microbial Control of Pests IOBC/WPRS". Rome 6-8.III.1989, pp. 23-25.

LIPA, J.J. 1990a. Lotta biologica nell Europa Orientale. In "Applicazioni Alternative nella Difesa delle Piante". Cesena, 3 giugno 1989 (ed. K.V. Deseo). Agro-Bio-Frut. Cesena, pp. 139-148

LIPA, J.J. 1990b. Update: Microbial pest control in Eastern Europe. The IPM Practitioner **12(2)**: 1-5.

LIPA, J.J. 1991. Microbial pesticides and their use in the EPRS-IOBC Region (Eastern Europe). IOBC/WPRS Bulletin **14(1)**: 23-32.

NOWAKOWSKI, L. 1977. Die Copulation bei einigen Entomophthoreen. Bot. Ztg. **35**: 217-222.

NOWAKOWSKI, L. 1883. Entomophthoreae. Contribution to the knowledge of parasitic fungi causing epizootics of insects. Pam. Akad. Umiej. Kraków, Wydz. Mat.-Przyr. (Memoires PAU) **8**:153-179.

SIEMASZKO, W. 1937. [Studies on Entomogenous Fungi of Poland]. Archives de Biologie de la Societe des Sciences et des Lettres de Varsovie **6(1)**: 1-93.

SIMMONDS F.J. 1958. Mutual cooperation. pp. 601-604. Transactions of the First International Conference of Insect Pathology and Biological Control. August 13 - 18, 1958. Prague (Czechoslovakia). Prague, 653 pp.

WEISER, J., HUBA, A. 1958. Zur Frage der internationalen Zusammenarbeiten auf dem Gebiet der biologischen Schädlingsbekämpfung. pp. 597-599. In „Transactions of the First International Conference of Insect Pathology and Biological Control". August 13 - 18, 1958. Prague (Czechoslovakia). Prague, 653 pp.

ANNEX I

PAPERS CONCERNING INSECT DISEASES AND MICROBIAL CONTROL PUBLISHED IN *INFORMATSIONNY BYULLETEN VPS MOBB* (INFORMATION BULLETIN EPS IOBC) NOS. 1-31 DURING 1980-1995

(All papers are in Russian with short English summaries)

ADAM, H. 1983. Missions and experimental work on joint application of *Encarsia* parasite and *Aschersonia* entomopathogenic fungi for biological control of *Trialeurodes vaporariorum* on cucumbers in glasshouses. *Information Bulletin EPS IOBC*, No **9**: 50-52.

ANONYMOUS, 1982. Meetings of EPS IOBC Standing Committees: Standing Committee on Microbiological Means of Plant Protection. *Information Bulletin EPS IOBC*, No **5**: 61.

ANONYMOUS, 1985. EPS IOBC Standing Committee Session on Microbiological Agents for Plant Protection. *Information Bulletin EPS IOBC*, No **13**: 79-83.

ANONYMOUS, 1987. Symposium: Microbiological method of colorado beetle in integrated system of plant protection (USSR, 1985). *Information Bulletin EPS IOBC*, No **18**: 76-78.

AZIZBEKJAN, R.R. 1993. Gene-engineering aspects of studying for *Bacillus thuringiensis*. *Information Bulletin EPS IOBC*, No **30**: 63-73.

BAICU, T. 1985. Compatibility of microbiological and chemical preparations applied in plant protection. *Information Bulletin EPS IOBC*, No **11**: 59-76.

BAICU, T. 1986. The toxicity of pesticides to entomophages and entomopathogenes used in integrated control systems. *Information Bulletin EPS IOBC*, No **14**: 47-73.

BAICU, T. 1993. Integrated pest management in the orchards in EPS IOBC. *Information Bulletin EPS IOBC*, No **29**: 7-21.

BAICU, T., GUSSEIN, S.M., GOGOACHA, K. 1987. Mixtures of pesticides with preparations based on *Bacillus thuringiensis* and their effectiveness against Colorado beetle. *Information Bulletin EPS IOBC*, No **18**: 60-66.

BAJAN, Tz., FEDORKO, A., KMITOWA, K. 1987. Experimental application of biopreparation for Colorado beetle control. *Information Bulletin EPS IOBC*, No **18**: 32-34.

BEDNAREK, A. 1987. The use of nematodes for Colorado beetle control. *Information Bulletin EPS IOBC*, No **18**: 52-54.

BEGLYAROV, G.A., SHUMILOV, V.A., DEVYATKINA, G.A., PONOMARYOVA, I.A. 1988. A new technology of culturing fungus *Verticillium lecanii* Zimm. and its effectivity for control of aphids under glasshouse conditions. *Information Bulletin EPS IOBC*, No **23**: 59-64.

BIACHE, G., CHAUFAUX, J. 1985. Mass rearing, formulations and use of baculoviruses. *Information Bulletin EPS IOBC*, No **12**: 76-84.

CHUKHRII, M.G., VOLOSHCHUK, L.F. 1993. The prospects for the production of viral entomopathogenic preparations and the development of a new standard „Methods to determine quality of viral insecticides". *Information Bulletin EPS IOBC*, No **30**: 33-39.

COULSON, J. 1993. International programme for biological method of insect pests control (Presentation made at V Session of EPS IOBC General Assembly). *Information Bulletin EPS IOBC*, No **30**: 10-14.

CROISIER, G. 1985. Identification and genetics of baculoviruses. *Information Bulletin EPS IOBC*, No **12**: 56-63.

DEDRYVER, C.A. 1985. Microbiological control of *Macrosiphum euphorbiae* Thomas, *Nasonovia ribis nigri* Mosl. and *Aulacorthium solani* Kltb. in greenhouses with the *Entomophthora* fungi. *Information Bulletin EPS IOBC*, No **12**: 23-31.

FEDORKO, A., BAJAN, S., KMITOWA, K. 1987. Entomopathogenic fungi as Colorado beetle repellent. *Information Bulletin EPS IOBC*, No **18**: 49-51.

FERRON, P. 1985. Artificial reproduction of *Beauveria brongniartii* in the population of *Melolontha melolontha* L. (Coleoptera, Scarabaeidae). *Information Bulletin EPS IOBC*, No **12**: 6-11.

FILIPPOV, N.A. 1987. The main ways of investigation concerning Colorado beetle biocontrol on yearly-ripening potato and eggplants. *Information Bulletin EPS IOBC*, No **18**: 67-71.

FILIPPOV, N.A. 1988. The present state and prospects of biological control of pests and plant diseases under greenhous conditions in the USSR. *Information Bulletin EPS IOBC*, No **23**: 7-12.

GORAL, V. M., LAPPA, N. V. 1995. Integrated protection of hothouse crops. *Information Bulletin EPS IOBC*, No **31**: 139-140.

GULY, V.V., LESKOVA, P.Ya., MURZA, V.I., SHETERNSHIS, M.V., IVANOV, G.M. 1986. The problems of microbiological product safety for environment and human health. *Information Bulletin EPS IOBC*, No **17**: 19-45.

HOSTOUNSKY, Z. 1987. Microsporidia as a limiting factor for Colorado beetle population number. *Information Bulletin EPS IOBC*, No **19**: 47-57.

KANDYBIN, N.V. 1985. Perspectives for the use of actinomyces producers of biologically active substances in plant protection. *Information Bulletin EPS IOBC*, No **12**:12-15.

KANDYBIN, N.V. 1986. On the IV Meeting of the IOBC RPS Standing Committee on the Microbiological Control Agents and Symposium on the Microbiological Control of the Colorado potato beetle in Integrated Systems of Plant Protection. *Information Bulletin EPS IOBC*, No **15**: 57-58.

KANDYBIN, N.V., KOVACHEVA, T.S. 1989. *Bacillus sphaericus* as a larvicidae (review). *Information Bulletin EPS IOBC*, No **27**: 15-29.

KANDYBIN, N.V., SHEKHURINA, T.A. 1983. Technology of use of microbial preparations for plant protection and its development. *Information Bulletin EPS IOBC*, No **6**: 27-41.

KANDYBIN, N.V., SMIRNOV, O.V. 1987. Problems and prospects for the use of *Bacillus thuringiensis* H-14 to control bloodsucking mosquitos and black fly. *Information Bulletin EPS IOBC*, No **21**: 6-31.

KANDYBIN, N.V., STUS, A.A. 1987. Ecological principles of Bitoxibacillin application against Colorado beetle. *Information Bulletin EPS IOBC*, No **18**: 7-12.

KEISER, L.S., KAPLAN, P.B., MONASTYRSKY, A.L., YAZLOVETSKY, I.G. 1989. The cabbage moth nuclear polyhedrosis virus accumulation depending on the lipid composition of larvae and their food. *Information Bulletin EPS IOBC*, No **28**: 81-87.

KOROL, I.I., ROMANOVETZ, Z.A. 1987. Microbial preparation used for Colorado beetle control and major criteria for estimation of their efficacy. *Information Bulletin EPS IOBC*, No **18**: 35-40.

KOZAR, Ph., SHIMON, E., ILOVAY, Z. 1989. Historical review of the biological plant protection development in the Hungarian People's Republic. *Information Bulletin EPS IOBC*, No **26**: 7-17.

LAPPA, N.V., GORAL, V.M. 1985. The use of Boverin in plant protection in the USSR. *Information Bulletin EPS IOBC*, No **12**: 47-51.

LAPPA, N.V., GORAL, V.V., ANOCHINA, V.P. 1988. Comparative action of entomopathogenic fungi on the glasshouse whitefly. *Information Bulletin EPS IOBC*, No **23**: 43-47.

LIKHOVIDOV, V.Y., GULYI, V.V., RADUL, M.M., RIBINA, S.Y. 1987. Receptivity of various ecological populations of Colorado beetle to microorganisms and biopreparations. *Information Bulletin EPS IOBC*, No **18**: 41-44.

LIPA, J.J 1983. Use of antibiotics and avirulent strains of pathogens to control plant diseases. *Information Bulletin EPS IOBC*, No **7**: 63-67.

LYSENKO, O. 1982. Main principles of entomopathogene registration and evaluation. *Information Bulletin EPS IOBC*, No **5**: 17-19.

LYSENKO, O. 1985. Taxonomy and diagnostics of *Bacillus thuringiensis*. *Information Bulletin EPS IOBC*, No **13**: 7-17.

MEZHEEVSKA, E., KMITOWA, K., BAJAN, Tz., FEDORKO, A. 1987. Infection of insects with mono- and polysporous cultures of entomopathogenic fungi. *Information Bulletin EPS IOBC*, No **18**: 45-48.

MURZA, V.I. 1987. Health safety problems concerning application of biopreparations for Colorado beetle control. *Information Bulletin EPS IOBC*, No **18**: 72-75.

ORLOVSKAYA, Ye.V. 1989. The use of viruses against insects injuring deciduous and coniferous trees in the USSR. *Information Bulletin EPS IOBC*, No **27**: 69-72.

PAVLUSHIN, V.A., AVANESOV, S. 1988. Prospects for application of entomopathogenic fungi against the greenhouse whitefly and aphids. *Information Bulletin EPS IOBC*, No **23**: 32-37.

PETRUHKINA, M.T. 1985. The use of antibiotic to control diseases of agricultural crops. *Information Bulletin EPS IOBC*, No **12**: 23-31.

RIBA, G. 1985. Genetic improvement of entomopathogenous Hyphomycetes. *Information Bulletin EPS IOBC*, No **12**: 32-40.

RIBINA, L.M., KOLCHEVSKY, A.G., PAVLYUSHIN, V.A., YUREVICH, I.A. 1987. Grounds for Bitoxibacillin application against Colorado beetle on potato. *Information Bulletin EPS IOBC*, No **18**: 13-19.

SAMOYLOV, Ju. K., KOSOY, S. M. 1995. Regional of the small-tonnage mirobiological means industry. *Information Bulletin EPS IOBC*, No **31**: 52-54.

SHEKHURINA, T.A. 1985. Forecasting virus epizooties of insect pests to reduce chemical treatments. *Information Bulletin EPS IOBC*, No **12**: 69-75.

SHTERNSHIS, M.V., GULII, V.V. 1985. The use of antioxidants as ingredients of the bacterial entomopathogenic preparations. *Information Bulletin EPS IOBC*, No **10**: 43-48.

SIKURA, A.I., SIKURA, L.V. 1987. The efficacy of *Bac. thuringiensis* subsp. *thuringiensis* preparation used for Colorado beetle control. *Information Bulletin EPS IOBC*, No **18**: 20-27.

STARCHESKY, I. P. 1995. Growth of the biological control means industry. *Information Bulletin EPS IOBC*, No **31**: 46-51.

STROEVA, I.A., KORNILOV, V.G. 1988. The efficacy of bacterial preparations for the pests control under greenhouse conditions. *Information Bulletin EPS IOBC*, No **23**: 79-83.

TARASEVICH, L.M., GULII, V.V. 1985. The state of affairs and perspectives for the use of entomopathogenic viruses in plant protection. *Information Bulletin EPS IOBC*, No **12**: 64-68.

TEPLYAKOVA, T.V, GUSHCHIN, F.L. 1985. The problems of the use of predaceous nematophagous fungi. *Information Bulletin EPS IOBC*, No **12**: 52-55.

VESELY, D. 1982. Mycoparasitism and potentialities for its use in plant protection from diseases. *Information Bulletin EPS IOBC*, No **5**: 11-16.

VIDENOVA, E.S. 1989. Perspectives of antibiotic application for plant protection. *Information Bulletin EPS IOBC*, No **27**: 7-14.

VORONINA, E.G. 1989. Entomophthoroses of aphids on different crops and forecastings. *Information Bulletin EPS IOBC*, No **27**: 30-68.

VORONTZOV, A.I. 1986. The present status and prospects for future development of biological control in forest protection. *Information Bulletin EPS IOBC*, No **14**: 39-46.

WEISER, J. 1983. To the problem of entomopathogene introduction. *Information Bulletin EPS IOBC*, No **9**: 53-54.

WEISER, J. 1983. Microbial insecticides: present status and prospects of use. *Information Bulletin EPS IOBC*, No **6**: 17-26.

Weiser, J. 1987. The efficacy of *Rhabdionvirus oryctes* used for Colorado beetle control. *Information Bulletin EPS IOBC*, No **18**: 55-57.

WEISER, Y., VIDENOVA, E., KANDYBIN, N.V., SMIRNOV, O.V. 1986. Technical characteristics and standardization of microbial entomocidal preparations. *Information Bulletin EPS IOBC*, No **16**: 44-52.

WEISER, J. 1987. Application of Boverol for Colorado beetle and other pests control. *Information Bulletin EPS IOBC*, No **18**: 58-59.

YARNIKH, V.S., TONKONOZHENKO, A.P., KATZ, M.B. 1987. Prospects for the use of *Bacillus thuringiensis* Berliner exotoxin producing strains aimed on the development of an effective products for Colorado beetle control. *Information Bulletin EPS IOBC*, No **18**: 28-31.

ZURABOVA, E.V. 1986. Efficient entomopathogenic preparation - Lepidocid: its production and use. *Information Bulletin EPS IOBC*, No **16**: 37-43.

ZURABOVA, E.R. 1987. Characteristicts of crystal-forming bacilli, occuring in host insects. *Information Bulletin EPS IOBC*, No **19**: 42-46.

ANNEX II

MAIN LITERATURE CONCERNING HISTORY AND DEVELOPMENT OF INSECT PATHOLOGY AND MICROBIAL CONTROL IN THE IOBC/EPRS REGION

ANONYMOUS, 1959. Biologicheskii Metod Borby s Vreditelyami Rastenii. Trudy Sessii. Kiev 1-4.IV.1958. [Biological Control of Plant Pests. Proceedings of the Conference. Kiev 1-4.IV.1958]. IUASN, Kiev 231 pp.

ANONYMOUS, 1968. Biologicheskij Metod Borby s Vrediteljami Rastenii [Biological Control of Plant Pests]. Zinatie, Riga 354 pp.

ANONYMOUS, 1973. Antibiotiki v Borbe s Boleznyami Selskokhozyastvennykh Kultur, Bakterialnye i Entomopatogennye Preparaty [Antibiotics in Control of Diseases of Agricultural Crops; Bacterial and Entomopathogenic Preparations]. VNIIBakpreparat, Moskva, Vol. **2**, 118 pp.

ANONYMOUS, 1974. Patologiya Chlenistonogikh i Biologicheskie Sredstva Borby s Vrednymi Organizmami. Tezisy Dokladov Pervoy Kievskoy Gorodskoy Konferentsii. [Arthropod Pathology and Biological Means to Control Noxious Organisms. Abstracts of First Kiev City Conference]. UNIIZR, Kiev, 201 pp.

ANONYMOUS, 1987. Entomopatogennye Bakterii i ikh Rol v Zashchite Rastenii [Entomopathogenic Bacteria and Their Role in Plant Protection]. VASKHNIL, Novosibirsk, 136 pp.

ANONYMOUS, 1975. Mikrobiologicheskie Sredstva Zashchity Rastenii i Bakterialnye Preparaty [Microbiological Means of Plant Protection and Bacterial Preparations]. Trudy VNIIBakpreparat (Moskva), Vol. **3**:1-168.

ANONYMOUS, 1975. VIII International Plant Protection Congress. Reports and Informations. Section V. Biological and Genetic Control. Moscow, 225 pp.

ANONYMOUS, 1978. Abstracts of the Second International Colloquium on Invertebrate Pathology and XIth Annual Meeting of the Society for Invertebrate Pathology. September 11-17, 1978. Prague, Czechoslovakia, 151 pp.

ANONYMOUS, 1980. Mikrobiologicheskie Sredstva Zashchity Rastenii i Bakterialnye Preparaty. Trudy. [Microbiological Means of Plant Protection and Bacterial Preparations]. Moskva, 168 pp.

ANONYMOUS, 1981. Kristalloobrazuyushchie Mikroorganizmy i Perspektivy ikh Ispolzovaniya v Ptitsevodstve [Cristalliferous Microorganisms and Perspectives of their Use in Chicken Husbandry]. ILIM, Frunze, 120 pp.

ANONYMOUS, 1981. Virusy Nasekomykh i Perspektivy ikh Prakticheskogo Ispolzovaniya v Zashchite Rastenii ot Vrediteley v Stranakh-Chlenakh VPS/MOBB [Insect Viruses and Perspectives of their Practical Use for Protection of Plants against Pests in Member Countries EPRS/IOBC]. Symposium Proceedings 19-21.XI.1980. Moskva, 144 pp.

ANONYMOUS, 1986. Mykopreparaty Ceskoslovenske Vyroby a Jejich Vyuzitu v Ochrane Polnich Kultur [Czechoslovak Mycopreparations and Their Use in Field Crop Protection. Proceedings of the First Seminar on Biotechnology in Integrated Pest Management]. Praha, 64 pp.

ANONYMOUS, 1986. Puti Sovershenstvovaniya Mikrobiologicheskoy Borby s Vrednymi Nasekomymi i Boleznyami Rastenii. Vsesoyuznaya Konferentsya 13 -15 May 1986, Velegozh. Tezisy Dokladov i Stendovykh Soobshchenii. [Ways of Improvements of Microbial Control of Noxious Insects and Plant Diseases. All-Union Conference, May 13-15, 1986, Velegozh. Abstracts of papers and posters]. Obolensk, 228 p.

ANONYMOUS, 1994. Tekhnologii Polucheniya Rasteniovodskoy Produktsii. RASN. Materialy Vserossijskogo Nauchno-Proizvodstvennogo Soveshchania. Krasnodar 24-26 Avgusta 1994. Pushchino, Part 1, [The Ecologically Safe and Pesticide-Free Technologies of Plant-Growing Production Obtaining. The Materials of the All-Russian Scientific-Production Conference. Krasnodar, August 24-27, 1994]. Pushchino, Part 1, 178 pp.

BOROVIK, R.V. (Ed.). 1989. Problemy Sozdaniya i Primeneniya Mikrobiologicheskikh Sredstv Zashchity Rastenii. 16-18 Maya 1989, Velehozh. Vsesoyuznaya Konferentsya - Tezizsy Dokladov. [Problems of Development and Application of Microbial Means of Plant Protection. May 16-18, 1989, Velegozh. All-Union Conference. Abstracts of Papers]. Moskva, Part 1, 1-170; Part II, 171-337.

CHUKHRII, M.G. 1982. Ultrastruktura Virusov Cheshuekrylykh-Vreditelei Rastenii [Ulstrastructure of Viruses of Lepidopterans Noxious to Plants]. Shtinitsa, Kishinev, 151 pp.

CHUKHRII, M.G. 1988. Biologiya Bakulovirusov i Virusov Tsitoplazmaticheskogo Poliedroza [Biology of Baculoviruses and Cytoplasmic Polyhedrosis Viruses]. Shtinitsa, Kishinev, 239 pp.

DIKASOVA, E.T. 1969. Granulez Ozimoy Sovki i Ego Primenenie dlya Borby s Etim Vreditelem [Granulosis Virus of Winter Cutworm and Its Application to Control This Pest]. FAN, Tashkent, 145 pp.

EVLAKHOVA, A.A. 1974. Entomopatogennye Griby [Entomopathogenic Fungi]. Nauka, Leningrad, 260 pp.

EVLAKHOVA, A.A., Shvetsova, O.I. 1953. Nastavlenie po Izuchenii Boleznej Nasekomykh i Primenenie Mikrobiologicheskogo Metoda Zashchity Rastenii [Recommendations for Studies of Insect Diseases and Application of Microbial Control in Plant Protection]. AN SSSR, Moskva-Leningrad, 80 pp.

GULII, V.V., IVANOV, G.M., SHTERNSHIS, M.V. 1982. Mikrobiologicheskaya Borba s Vrednymi Organizmami [Microbiological Control of Noxious Organisms]. Kolos, Moskva, 272 pp.

GULII, V.V., RYBINA, S.Yu. 1988. Virusnye Bolezni Nasekomykh i ikh Diagnostika [Virus Diseases of Insects and Their Diagnostic]. Shtinitsa, Kishinev, 124 pp.

ISSI, I.V., LIPA J.J. 1968. Report on identification of Protozoa pathogenic for insects in the Soviet Union (1961-1966). Acta Protozoologica **6**: 281-290.

ISSI, I.V. 1986. Mikrosporidii kak tip parazitichеskikh prosteyshikh [Microsporidia as a Phylum of Parasitic Protozoa]. Mikrospriidii: Protozoologiya, Vol. **10**, pp.6-136.

KANDYBIN, B.V., BARBASHOVA, N.M., SMIRNOV, O.V. 1980. Katalog Entomopatogenennykh Mikroorganizmov Deponirovannykh v uchrezhdeniakh - sotrudnikakh Vostochnopalearkticheskoj Sektsii MOBB [A Catalogue of Entomopathogenic Microorganisms Deposited in Institutions Collaborating with Eastern Palearctic Section IOBC]. Praga (Czechoslovakia), 81 pp.

KANDYBIN, N.V. 1989. Bakterialnye Sredstva Borby s Gryzunami i Vrednymi Nasekomymi. Teoriya i Praktika [Bacterial Means to Control Rodents and Noxious Insects: Theory and Practice]. Agropromizdat, Moskva, 172 pp.

KOK, I.P. SKURATOVSKAYA, I.N, STROKOVSKAYA, L.I. 1980. Molekularnye Osnovy Reproduktsii Bakulovirusov [Molecular Bases of Reproduction of Baculoviruses]. Naukova Dumka, Kiev, 176 pp.

KOVAL, E.Z 1974. Opredelitel Entomofilnykh Gribov SSSR [Key for Identification of Entomophilic Fungi of the USSR]. Naukova Dumka, Kiev, 259 pp.

KOVAL, E.Z. 1984. Klavicipitalnye Griby SSSR [Clavicipitales Fungi of USSR]. Naukova Dumka, 287 pp.

LIPA, J.J. 1974. An Outline of Insect Pathology. USDA/NSF. Warsaw, 269 pp.

MEKHTIEVA, N.A. 1979. Khishchnye Nematofagovye Griby-Gifomitsety [Predatory Nematophagous Hyphomycetous Fungi]. Elm, Baku, 245 pp.

POLTEV, V.I. 1963. Mikrobiologicheskie Metody Borby s Vrednymi Nasekomymi [Microbiological Control Methods against Noxious Insects]. AN SSSR, Moskva, 134 pp.

POLTEV, V.I. 1969. Mikroflora Nasekomykh [Insects Microflora]. SO Nauka, Novosibirsk, 271 pp.

Progress in Invertebrate Pathology 1958-1978. Proceedings of the International Colloquium on Invertebrate Pathology and XIth Annual Meeting Society for Invertebrate Pathology. September 11-17, 1978. Prague, Czechoslovakia, 265 pp.

ROMASHEVA, L.F., BALYKIN, A.V., VIDOMSKII, E.V., KRYLOVA, V.B. 1976. Mikrobiologicheskie Metody Borby s Argasovymi Kleshchami [Microbiological Control Methods Against Argasid Ticks]. ILIM, Frunze, 99 pp.

SHTERNSHIS, M.V. 1988. Mikrobiologicheskaya Borba s Vreditelyami Selsko-Khozyastvennykh Kultur Sibiri i Dalnego Vostoka [Microbiological Control with Agricultural Pests of Siberia and Far East]. Rosagropromizdat, Moskva, 125 pp.

SHUMAKOV, E.M., GUSEV, G.V., FEDORINTCHIK, N.C. 1975. Biologicheskie Sredstva Zashchity Rastenii [Biological Means of Plant Protection]. Kolos, Moskva, 416 pp.

SIDELNIKOV, K.E., OREKHOV, D.A., KARTOMYSHEVA, O.P, PILIPENKO, A.A., MOROZOV, O.V. 1994. Preparaty dlya Zashchity Rastenii [Preparations for Plant Protection]. Kolos, Moskva, 319 pp.

SONIN, M.D. 1985. Helminths of Insects [Gelminty Nasekomykh]. Amewrinf Publ. Co., New Dehli, 227 pp.

TALALAEV, E.V. 1965. Ispolzovanie Mikroorganizmov dlya Borby s Vrednymi Nasekomymi Lesov Vostochnoy Sibiri [Use of Microorganisms to Control Noxious Insects in Forests of Eastern Siberia]. Izvestiya Biologo-Geograficheskogo Nauchno-Isledovatelskogo Instituta pri Irkutskom Gosudarstvennym Universitete im. Zhdanova (Irkutsk) **19**(1): 1-100.

TARASEVICH, L.M. 1975. Virusy Nasekomykh [Insect Viruses]. Nauka, Moskva, 198 pp.

VOROBEVA, N.N. (Ed.). 1974. Virusy Nasekomykh [Insect Viruses]. Nauka, Novosibirsk, 138 pp.

VOROBEVA, N.N. 1976. Entomopatogennye Virusy [Entomopathogenic Viruses]. Nauka, Novosibirsk, 286 pp.

WEISER, J. 1966. Nemoci Hmyzu [Diseases of Insects]. Academia, Praha, 554 pp.

WEISER, J. 1974. An Atlas of Insect Diseases. 2nd Edition. Academia, Prague, 240 pp.

The IOBC/WPRS working group on insect pathogens

Peter H. Smits

DLO - Research Institute for Plant Protection (IPO-DLO)
POB 9060, 6700 GW Wageningen, The Netherlands

The start

The IOBC/WPRS working group on insect pathology was started in 1985 on the initiative of Chris Payne. Jürg Huber was convener from 1989 to 1991.
Since 1991 Peter Smits is convener of the group.

The working group was founded because it was felt there was a need for a forum for scientists in the field of insect pathology in Europe. Many insect pathologists already participated and still participate in other working groups of IOBC/WPRS. A forum, in the form of bi-annual meetings and more specialised workshops, would allow the exchange of information and stimulate contacts and cooperation. The first meetings were organised in 1987 (Versailles) and 1989 (Rome) and were each attended by seventy persons. From there the group has grown to about 110 attendants per meeting with another 50-60 interested in attending the meetings. This covers the majority of people working in this field in Western Europe. More and more researchers from Eastern European countries attend the meetings as well. In 1989 Jürg Huber succeeded Chris Payne as convener. When he stepped back as convener in 1991, due to other commitments within IOBC/WPRS, Peter Smits became convener of the working group.

Bi-annual Meetings

The working group has organized 5 bi-annual meetings:

1987 Versailles, France
1989 Rome, Italy
1991 Wageningen, The Netherlands
1993 Zurich, Switzerland
1995 Poznan, Poland

and several workshops on entomopathogenic nematodes and fungi.
There are 100-120 scientists that regularly attend the meetings of the working group.

The 4-day meetings are generally organised around a theme, but there is also room for contributed papers on various subjects. The bi-annual meetings gradually get the status of a conference rather than a discussion meeting, although the balance is kept by splitting the group, at least for part of the meeting, into subgroups on nematodes, fungi, bacteria and viruses. By limiting the number of papers and extending the time per paper and by putting additional focus on

posters, that are all discussed by small groups, we have been able to create and maintain a very open and loose atmosphere that stimulates discussion and mutual contact between individual members.

In February 1991 the third bi-annual meeting of the working group in Wageningen, organised by Peter Smits, was attended by 110 participants. The programme consisted of presentations, posters and discussion meetings during 3 days. The special topics for the meeting were "Diagnosis of insect diseases, with particular emphasis on neglected groups of micro-organisms" and "Behaviour of insect pathogens in the environment, i.e. their persistence, ecology and epizootiology." Furthermore, in two parallel sessions, the insect mycologists and their colleagues working on insect parasitic nematodes discussed their specific problems and organised themselves in two formal subgroups of the working group. Bernard Papierok and Ralf-Udo Ehlers were chosen as conveners of these two groups.

In May 1992 a workshop on "Nematode Taxonomy" was organised in Raisdorf (Kiel, Germany) by R.U. Ehlers. The workshop was attended by 50 scientists from Europe. Both methods based on morphological characteristics as techniques for DNA-analysis were demonstrated.

In September 1993 a 4-day general meeting was held in Zurich, organised by Prof. Georg Benz, that was attended by 117 scientists. The meeting was directly followed by a 1-day workshop on Entomopthorales organised by Siegfried Keller. At the meeting 78 papers or posters were presented on insect pathogenic bacteria, viruses, fungi, nematodes and protozoa. Special themes at the meeting were "insect pests difficult to control with microbials" and "interaction between pathogens and the host defense mechanisms".

In October 1993 a workshop was organised by Ann Burnell a.o. in Maynooth, Ireland. Over 60 people attended the 4-day meeting. Main themes were: genetics of insect parasitic nematodes, cryopreservation techniques, symbiotic bacteria, bioassays and DNA-analysis using PCR and Rapids.

Structure of the working group

Working Group convener:	Peter Smits
Subgroup Insect pathogenic Nematodes:	Ralf-Udo Ehlers
Subgroup Insect pathogenic Fungi:	Bernard Papierok

In May 1990 the scientific committee met in Darmstadt to discuss the future activities of the working group and the programme for the meeting in Wageningen. The main conclusions of this meeting were: 1. To form two subgroups: one on Nematodes and on Fungi. 2. To continue the efforts to make a directory. 3. The continue our efforts to create a database on insect viruses. 4. To create possibilities within the group for identification of pathogens.

In 1991 Jürg Huber stepped back as convener, due to his commitments as treasurer of IOBC/WPRS. Peter Smits was appointed convener of the working

group at the Wageningen meeting in February 1991. At this meeting Ralf-Udo Ehlers was appointed convener of the subgroup Nematodes and Bernard Papierok was appointed as convener of the subgroup Fungi.

The main aims of the working group

A. To provide a forum for the exchange of information in the field of microbial control of insect pests. This is done through the organisation of bi-annual general meetings and specialised workshops.

B. To stimulate collaborative research between members of the working group.

C. To stimulate research, commercial development, registration and use of microbial control agents.

Novel ecologically safe biopesticides against insects and mites

N.V. Kandybin, O.V. Smirnov

All-Russian Research Institute for Agricultural Microbiology, Podbelskogo, 3, 189620, St.-Petersburg - Pushkin, Russia

Summary

New investigations of ARRI for Agricultural Microbiology broadens the spectrum of microbial pesticides. Among then: Bactoculicide (based on Bt) against blood sucking mosquitoes and certain plant pest, Bacicole with specific action against harmful Coleoptera and Actinine (based on *Streptomyces)* for spider mite control in greenhouses. The introduction of these preparations leads to a decrease of the chemical pollution of the environment.

Introduction

The widening spectrum of new microbial means for pests control helps to improve the ecological crisis situation in the world. Nowadays, because of various reasons, microbial pesticides are being used considerably less than is desirable but also considerably less than is possible, having in mind the modern level of biotechnological development. In addition there are no alternative pesticides to solve the great number of difficulties in creating entirely "pesticide free" technologies for cultivating and protecting practically every agricultural crop.

The main objective in creating new preparations is to obtain an effective, technological and stable (as far as its characteristics are concerned), microbial product, which guarantees safety for man and non-target species. The stages in the production development are as follows:

1) the studying of the physiological properties of microorganisms and the assessment of its cultivation methods.

2) the creation of prescriptive application form adapted to the particulaties of a protected plant, work conditions and pest ecology,

3) the creation of preparation application technology in the context of a cultivating and protective system of any concrete crop.

All these important and scientifically important stages surely must come second when compared to the problem of isolating a microorganism that forms the basis for a new preparation. Screening and selection work in order to obtain a first product is followed by the strain evaluation as far as its safety for producers and consumers is concerned.

Everything mentioned above characterizes briefly the work that makes it possible to create some new preparations in ARRIAM.

Results and Discussion

Bactoculicide is a preparation on the basis of Bt subspecies *israelensis* for bloodsucking mosquitoes and blackfly control. The discovery of this subspecies made it possible to turn to the microbiological control for transmissive infection carriers. Earlier the control of harmful Dipteran larvae was carried out mainly by chemical larvicides (for example, phosphororganical) which greatly contaminate surroundings. However, the other preparations on the basic of Bt, Bactoculicide is safe for a man and warm blooded animals, harmless for fish and hydrobionts. Large scale trials and its application in Russia, states of UIS and in a number of foreign countries (Czechia, India, Shri-Lanka, Cuba and so on) showed its high efficiency in all ecologic-geographical zones and in various types of waterpools: natural and artificial. Total larvae mortality with Bactoculicide application dose of 1,0 - 1,5 kg/ha occurs 48-72 h after application.

Besides control of pests in cattle breeding and of dangerous human disease carriers (such as malaria, for instance), Bactoculicide finds its application in plant protection. It is very effective for control of *Cricotopus silvestris Fabr.* - a dangerous rice pest and for control of *Lycoriella fucorum Frey,* that injures fungi in commercial agaric mushroom culture, as well.

The other BT subspecies - *darmstadiensis* - is the base for a new preparation called Bacicol. This strain possesses specific activity against phytophagous Coleopteran pests. Bacicole is highly effective against Colorado potato beetle, cruciferous fleas, strawberry - raspberry weevil and against a number of other Coeleopteran species. Bacicole elaboration allows use in the pest control schemes of potato (Tab.1), tomato, cabbage, cereals, berry plantations with a new ecologically safe preparation. Bacicole, applied against cruciferous fleas, to protect cabbage, is well combined with BT preparations used against leafcutting *Lepidoptera* to protect the same culture.

Table 1. Field trails of Bacicole against Colorado beetle (larvae 1-2 instrars) on potato (Tiraspol).

Variants	Efficiency on 10-th day after treatment
Bacicole 0,4%	92,8
Bitoxybacilline 0,4%	90,1
Synthetic pyrethroid 0,02%	71,8
Control	Increase of population

In greenhouses spider mite control plays an important role. This pest, as many others, has become resistant to the application of chemicals. The preparation Actinine- it is elaborated in ARRAM - can solve the problem of spider mites.

One can judge the efficiency of Actinine by its application doses 30-50 g/ha (Tab.2). This preparation is created on the basis of *Streptomyces globisporus*, safe for man and warmblooded animals, mites - acariphagan and pollinators.

Table 2. Comparative effictivity of Actinine-M and another microbial acaricide in greenhouses against *Tetranichus urticae Koch*

Variants	Dose (kg/ha)	Effectivity (%)
Actinine M	0,052	98,3
Mycoafidine	15,0	82,3
Bitoxybacilline	20,0	91,3
Actelyc (chemical etalon)	3,0	89,1

Investigations on the technology of production and application, on the modernization of all new preparations and on the search for new spheres of their application are being conducted. The three biopreparations contribute significantly to the ecologization of plant protection. To expand their practical use simplified prescriptive forms are being made for these preparations in order to facilitate small scale regional production.

Conclusions

1. The new biopesticides created in ARRI for Agricultural microbiology are relevant for different areas of practical use.
2. There are two new preparations of BT:
   Bactoculicide - against harmful Diptera and Bacicol for control of Coleopteran pests.
3. Actinine - a bioacaricide based on certain *Streptomyces* is highly effective for spider mite control.

References

N.V. KANDYBIN, 1990. Ecological exnediency of the research and employment of the microbiological means for controlling the rodents and insects. Proceedings of the All-Union Research Institute for Agricultural microbiology "Microbiological aspects of environment protection under intensive agriculture", 60,121-130. (In Russian).

O.V. SMIRNOV, V.P. YERMOLOVA, A.A. STUS, 1990. Bactoculicide as a biological means for controlling the population of blood sucking mosquitoes. Proceedings of the All-Union Research Institute for Agricultural microbiology "Microbiological aspects of environment protection under intensive agriculture", 60,147-156 (In Russian).

Perspectives of Biological Control of Locusts

I.V. Issi

All-Russian Institute for Plant Protection, St.-Petersburg

Interest in the biological control of Orthoptera occured several times since the end of the 19-th century. As a rule investigations in this field stopped in the period between two infestations. New explosions of locusts stimulated further research. In the last years some success was obtained in the creation of new biological preparations against locusts. Their application on crops had rather good results. However a system of biological control of locusts still doesn't exist due to several reasons.

Our own investigations and the analysis of literature brought us to the conclusion that the principles of locust biocontrol should be changed. Locusts differ very essentially from traditional test-objects of microbiological control. Their biological characteristics, such as: (1) physiological alterations during the transformation from solitarious to gregarious phase; (2) swift migrations to new places; (3) settling in vast territories, are all factors which hamper successful application of entomopathogenic microorganisms in biological control. However very intimate relations of locusts and grasshoppers with wild nature and development near fresh water basins are favourable for the introduction of locust pathogens as biological control agents.

Our investigations of natural locust populations in different regions, with contrasting climate conditions: Uzbekistan, Yakutia, and Povolgie, revealed a suppression in the quantity of insects by a complex of various pathogenic microorganisms. Fungi are the most abundant among locust pathogens: more than 30 species were discovered. The percentage of insects infected by fungi was the highest too. High mortality was caused by entomopathogenic and phytopathogenic fungi species, mostly by *Entomophthora grylli, Beauveria bassiana* and *B. tenella.* Protozoa, such as *Microsporidia* and *Sarcodinae* were the second in frequency and pathogenicity. Bacteria (mostly *B. thuringiensis*) were rare (<1%). *Metarrhizium anisopliae* and viruses were not found.

Mass epizooties in populations of *Calliptamus italicus* in Povolgie and *Chortippus albomarginatus* in central Yakutia were caused by *E. grylli.* Microsporidian species and *Malpighamoeba locustae* were found mostly in Middle Asia and Siberia. Nematodes of the family Steinernematidae were found in Yakutia and in other regions. High percentage of insect mortality was caused by several phytopathogenic fungi (*Fusarium oxysporum* and other fungal species), which infected different organs of living insects. Most of the pathogenic microorganisms were not specific and possessed a wide host range. Microsporidian species, on the contrary, invaded only one or few Orthoptera hosts. Local forms of microorganisms were more effective, than the introduced ones.

We consider the following forms of entomopathogenes to be the most promising as a basis for future biopreparations: one strain of *B. tenella* developing at low humidity and a nematode (*Steinernematidae*), withstanding low temperature conditions. A strain of *B.tenella* was revealed in adults of *Dociostaurus maroccanus,* which is more xerophylic than other Orthoptera species. Greenhouse experiments in the southern regions of Russia showed its high effectiveness against sucking insects. Nematode species from Yakutia invaded its hosts at lower temperatures than other nematodes of the family of Steinernematidae. Field experiments on the application of biopreparations (bouverins, preparations from nematodes) showed good biological

effectiveness - 60-90% for non-migrating insects.

Summarizing this short communication, I would like to make the following points. At the moment we possess: (1) a broad list of microorganisms, that may be applied at various phases of locust development and on different levels of their density; (2) data on laboratory and field experiments of applications of microorganisms and nematodes, which are promising as a basis for biopreparations. We suggest that the emphasis in biological control of locusts should be made on preventive applications of biological preparations in initial reservation stations. In this case the area of application will be essentially smaller, than during the infestation period.

The main task is to prevent the locust aggregation and migration on crops after they reach high population densities. Such biological agents as fungi, microsporidians and nematodes may be introduced in the reservation areas every year or every other year.

Biological control of sciarid flies.

Rovesti L., Viccinelli R., Barbarossa B.

C.N.R. - Centro di Studio dei Fitofarmaci
Bologna - Italy

Summary

Steinernema feltiae, Bti and azadirachtin (neem) significantly reduced the number of adult flies emerging from the compost in mushrooms, their effectiveness lasting for at least 3 weeks. The treatments also reduced the number of damaged carpophores, though no statistical differences were recorded in the yield of mushrooms. On ornamentals, good control was achieved both in an experimental trial on primrose and in a commercial-type trial on begonia. However, the treatment of large potted poinsettias gave unsatisfactory results.

1. Introduction

Sciarid flies are economically important pests both in mushroom growing and in the production of seedlings and cuttings, where they cause significant economical losses. Chemical control of these flies is not always effective or practicable. Although no *ad hoc* studies have been carried out to demonstrate the presence of resistant populations of sciarids in Italy, several instances of partial or even total failure in the control of these insects using some chemicals have been observed in recent years under practical conditions. The interest for alternative control methods is increasing among the growers, both for the need to meet the ever-increasing demand for residue-free produces and for an increased awareness of the dangers associated with the intensive use of chemical pesticides in close environments such as greenhouses and mushroom houses. Both entomopathogenic nematodes and Bti have been shown to possess a good potential to control sciarid flies (Cantwell and Cantelo, 1984; Grewal and Richardson, 1993; Nickle and Cantelo, 1991; White and Jarrett, 1990). However, no biopesticides are registered in Italy for use against sciarid flies, while very little use is made of S. feltiae. For this reason, we tested these biorationals to assess their potential for use against sciarid flies under practical conditions both in mushrooms and in ornamentals.

2. Materials and methods

Trial on mushrooms. A small-plot trial was carried out against *Lycoriella* sp. infesting white button mushroom (*Agaricus bisporus*) cultivated in shelf-beds. Plots were 2 m^2 in size, replicated four times. Treatments were as indicated in tab. 1, and were applied on top of the casing layer using a watering can. The application was made 9 days after the casing with a layer of soil, to avoid possible damages to the nematodes by the formaldehyde solution routinely used

by the grower to sterilize the soil. At treatment, adults of the sciarid could already be seen in the mushroom house. In order to assess the efficacy of treatments, both emergence of adult flies from the plots and mushroom yield were checked weekly.

Trials on ornamentals. On primrose grown in trays (55x33 cm; 336 seedlings/tray) treatments were applied using a watering can. Plots were made up by two trays. The first application was made preventively approx. 10 days after seedlings had emerged from compost; The second treatment was applied about a month later, when infestation was already under way. Single plots were then caged to prevent reinfestation. Treatments were as indicated in tab. 2; their efficacy was assessed by checking both the number of flies emerging from the trays (1 yellow sticky trap (100 cm^2) was placed in each cage and replaced weekly) and the number of well developed plants at the end of the growing cycle.
On begonia grown under the same conditions, a commercial-type trial was also carried out. Unreplicated plots of 150 trays each were treated with *Steinernema feltiae, Bt israelensis*, carbofuran and water (control). Treatments were applied through the irrigation system, apart from granular carbofuran which was hand-distributed. The assessment was made on 12 trays/treatment, by checking at the end of the growing cycle the number of missing plants/tray and the presence of sciarid larvae on the surface of trays.
Finally, a trial on potted poinsettia (Ø of pots: 20 cm) was done comparing cyromazine, Bti and azadirachtin. Twenty pots were used/treatment, in groups of 5. Single plants were capped with plastic bags following treatment, and the number of flies emerging from each pot was checked every 2-3 days afterwards.

In all cases *Bradisia paupera* was the species present.

3. Results

Mushrooms
Tab. 1 - Adult *Lycoriella* sp. trapped/plot

treatment (a.i.)	rate /m2 g form. (g a.i.)	n° treats.	sampling date 7 d.a.t.	14 d.a.t.	21 d.a.t.
Armor (cyromazine)	2,5 (0,37)	1	338,5 B	198,0 C	117,5 C
Exhibit (S. feltiae)	- (500.000)	1	582,0 AB	394,5 BC	248,3 BC
Bactimos (Bti)	10 (-)	1	584,5 AB	386,8 BC	353,5 B
Neem-Azal (azadirachtin)	2 (0,2)	1	576,8 AB	490,8 AB	303,5 BC
Control	-	-	778,5 A	749,8 A	794,8 A

* Values followed by the same letter within each sampling are not significantly different at p=0.05 (Tuckey's test).

No statistically significant differences in the yield were recorded, but all treatments significantly reduced the number of mushrooms damaged by the sciarid larvae.

Ornamentals

Small-plot trial on primrose

a) No. of *Bradisia paupera* adults trapped/plot

treatment (a.i.)	rate /m2 g form. (g a.i.)	n° treats.	sampling time 12 d.a.t.	19 d.a.t.	25 d.a.t.
Trigard 75WP (cyromaz.)	0,2 (0,15)	2	7,75 AB	4,75 B	11,00 C
Exhibit F 27 (S. feltiae)	- (500.000)	2	10,00 AB	7,75 AB	19,75 BC
Bactimos (Bti)	10 (-)	2	5,00 B	6,00 B	11,75 C
Neem-Azal W (azadirach.)	2 (0,2)	2	7,50 AB	3,00 B	23,75 B
Control	-	-	12,75 A	12,75 A	36,00 A

* Values followed by the same letter within each sampling are not significantly different at p=0.05 (Tuckey's test).

b) Number of plants/tray

treatment	No. of plants
cyromazine	504,75 B
S. feltiae	527,50 A
Bti	507,00 B
azadirachtin	507,50 B
Control	500,25 B

Commercial-type trial on begonia

treatment (a.i.)	rate /m2 g form. (g a.i.)	n° treats.	Mean No. of plants missing/tray	mean No. of Bradisia larvae/tray
Exhibit F 27 (S. feltiae)	- (500.000)	1	5.70	0.40
Bactimos (Bti)	10 (-)	1	3.60	0.08
Marshall (carbofuran)	-	1	4.80	0.90
Control	-	-	5.20	2.30

Trial on potted poinsettia

Cyromazine and azadirachtin were the only treatments which reduced significantly the emergence of flies from treated pots, although sciarid control was far from complete. Bti-treated pots did not differ from the control.

4. Discussion and conclusions

All biorationals tested in our trials have shown potential for use against sciarid flies, both in mushrooms and in ornamentals, even when used in commercial-type applications. The control of sciarid flies appears therefore a very interesting niche market for these biological insecticides also under the cultivation conditions found in Italy. On mushrooms azadirachtin, *S. feltiae* and Bti significantly reduced the number of adult flies emerging from the compost, and their effect was evident for at least 3 weeks. With regard to mushroom yield no statistical differences were observed between the treatments, but presumably this was mostly due to the patchy emergence of the mushrooms on the beds and to the limited size of plots. Nevertheless, all treatments significantly reduced the amount of mushrooms damaged by sciarid larvae. The number of sciarids was similarly reduced on primrose and begonia. However, the treatment of relatively large pots gave unsatisfactory results for all treatments tested, including the chemical. A possible explanation for it could be that the top layer of the potting substrate acted as a filter, adsorbing most of the active ingredient(s) while most of the larvae developed in the bottom part of the pots. Further testing will be done in the future to assess the need for application methods other than drenching (e.g. incorporation in the compost).

5. References

CANTWELL, G.E. & CANTELO, W.W., 1984.Effectiveness of *Bacillus thuringiensis* var. *israelensis* in controlling a sciarid fly, *Lycoriella mali*, in mushroom compost. Journal of Economic Entomology, 77: 473-475.

GREWAL, P.S. & RICHARDSON, P.N., 1993. Effects of application rates of *Steinernema feltiae* (Nematoda: Steinernematidae) on biological control of the mushroom fly *Lycoriella auripila* (Diptera: Sciaridae). Biocontrol Science and Technology, 3: 29-40.

NICKLE, W.R. & CANTELO, W.W., 1991. Control of mushroom-infesting fly, *Lycoriella mali*, with *Steinernema feltiae*. Journal of Nematology, 23: 145-147.

WHITE, P.F. & JARRETT, P., 1990. Laboratory and field tests with *Bacillus thuringiensis* for the control of the mushroom sciarid *Lycoriella auripila*. Proc. Brighton Crop Protection Conference - Pests and Diseases, 1: 373-378.

APPLICATION OF BIOLOGICAL CONTROL AGENTS : SOME THEORETICAL CONSIDERATIONS OF DISPERSAL

Andrew C. Chapple

Ecogen Europe Srl., Parco Tecnologico Agro-Alimentare dell'Umbria,
Fraz. Pantalla 06050 TODI (PG), ITALY

Abstract

The dispersal of biological control agents (BCAs) is discussed, with respect to the two most commonly used atomisation systems in broad-acre agriculture - hydraulic flat fan nozzles and airblast sprayers - as well as spinning disc applicators. The effects of atomisation method, application volume, adjuvants, and the particle size of the BCA is discussed, with respect to nematodes and fungal spores. The efficiency of distribution and inefficiency of use of formulation components and spray adjuvants is also considered.

Introduction

The application of pesticides into broad-acre agriculture has long been widely recognised as being an extremely wasteful process (Graham-Bryce, 1983). Despite much research into alternative application methods (eg. Cooke *et al*, 1986), the hydraulic flat fan nozzle and the airblast sprayer remain the main means of introducing any crop pest control agent - chemical or biological - into a crop. In most cases, agricultural chemicals are not applied as particles in suspension but are emulsified, dispersed, or dissolved into the carrier spray volume. This is also true of most adjuvants and active formulation components. On the other hand, biological control agents (BCAs) are almost without exception particles: compared with the disolved, dispersed, or emulsified particle size of a modern agrochemical pesticide, BCAs have a relatively large particle size and hence are comparably few in number per unit volume of sprayed liquid.

The droplet spectra produced by sprayers can be modified by formulation (Krueger and Reichard, 1985), or spray tank additives (Chapple *et al* 1992: see Fig. 1), by changing the application volume, or varying the application parameters available for a particular device (eg. orifice size and pressure for hydraulic flat fans). It should be noted that the pattern of distribution by volume under hydraulic nozzles can also change, sometimes substantially (Chapple *et al*, 1992). Also, different application devices produce widely different droplet spectra (Fig. 2). The atomisation system can be considered a "sampling" system: different drop sizes sample different volumes from the spray tank. Any change in droplet spectra will alter the likelihood of the BCA being present in any given drop - a "useful" drop - and can severely affect the proportion of "useful" drops produced. Small drops, wide drop size ranges, and large spray volumes all contribute to a lower probability that a given drop will contain a particle (or particles) of the BCA. In general, for BCAs, there is no strict within-drop concentration effect as is found with agricultural chemicals or biorationals with a wide particle size range (eg. *Bt*). It is therefore more appropriate to consider the application system as a "delivery system" rather than an atomisation system: its efficiency can be expressed in terms of how well the particles are dispersed into the spray cloud (see Table 1, footnote '**d**').

Application volumes - ie. l/ha carrier liquid - in broad acre crops are normally around 200 l/ha, occasionally as high as 800 l/ha in tree and vine crops. In broad acre crops, application volumes >800 l/ha are relatively rare. It is arguable, and is a premise of this paper, that if BCAs are to succeed in broad acre agriculture, then they must be applied using conventional equipment and at standard application volumes. For example, entomopathogenic nematodes are routinely applied as a "drench" into pots for insect control (eg. black vine weevil). One litre of water added post application per 50 cm diameter pot is the equivalent of applying approximately one olympic-sized swimming pool per 44 ha, or

5.1 l/m2. By contrast, applying 200 l/ha, the application volume per unit ground area (ie. ignoring leaf area indices) is 0.02 l/m2. With the exception of protected and irrigated crops, expecting the grower to apply a BCA into broad acre agriculture in such high volumes is unrealistic.

In this paper, two BCAs are discussed, representing a wide range of particle size: the spores of *Ampelomyces quisqualis*, the fungal hyper-parasite of various powdery mildews[a], and entomopathogenic nematodes.

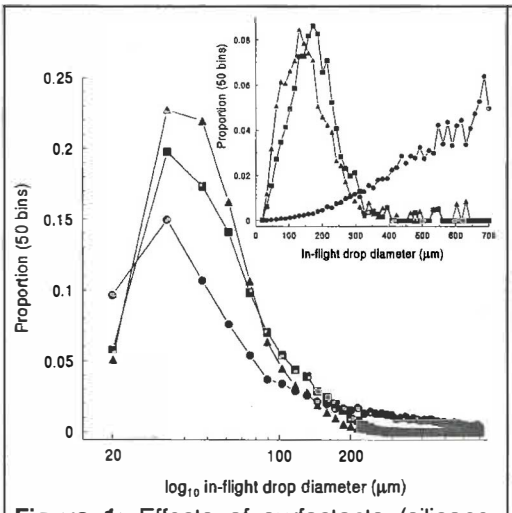


Figure 1: Effects of surfactants (silicone surfactant [triangles] and polymeric drift retardant [circles]) compared with water [squares] on the droplet spectra produced by hydraulic flat fan (Hardi 8020-10), by number (log_{10} in flight diameter) and by volume (inset: linear x-axis).

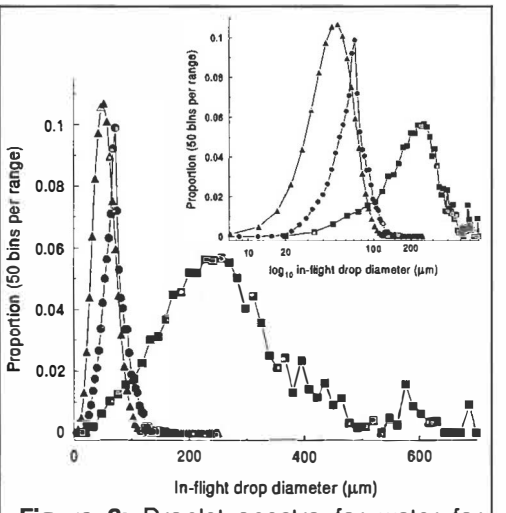


Figure 2: Droplet spectra for water for three atomisers : hydraulic nozzle (Hardi 8020-10 [squares]), Micron Ulva+ spinning disc (red restrictor, c. 6.2 l/ha [circles]), and airblast sprayer [triangles], by number and by volume (inset) respectively (Note: log_{10} scale on inset x-axis).

Nematodes.

The application of nematodes can largely ignore drop size: in general, nematodes are large and overcome atomisation. As a nematode reaches the edge of the sheet of liquid that is being broken up into drops, either the nematode will be included in any large enough drop that is being formed or the nematode itself will be a focus for drop formation. If a small drop was likely to be formed at that moment, then the nematode will cause a much larger drop to be formed, containing the nematode and carrying with it a quantity of formulation components and spray tank adjuvants, if any. Consequently, nematodes are deposited where the volume of applied liquid is found (see Fig. 3). However, applying 500,000 nem./m^2 (or 5 x 10^9/ha) at 242 l/ha and assuming that a nematode will "fit" into a minimum diameter drop of 300μm, then approx. 29% of the spray volume will be associated with the nematode (for calculation, see Appendix). Or, put another way, approx. 70% of the formu-

a *A. quisqualis* is currently being commercialised in the USA (and, shortly, Europe) as AQ_{10}(R) by Ecogen Inc., Langhorne, PA, USA. Entomopathogenic nematodes are currently being commercialised as various products based mainly on the two genera *Heterorhabditis* and *Steinernema*.

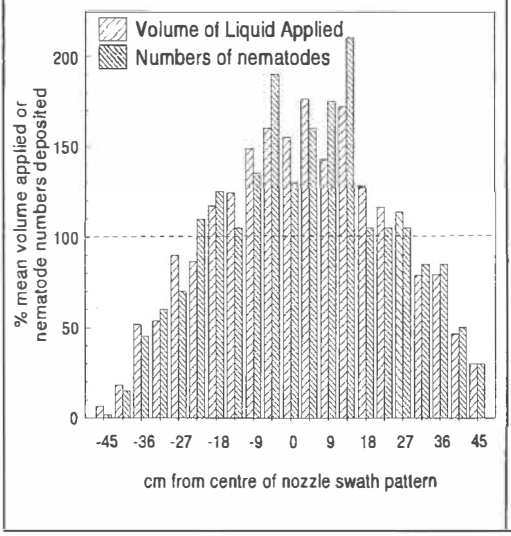


Figure 3: Distribution of nematodes with respect to distribution of volume applied by the nozzle (TeeJet XR8002VS nozzle). (Data from COST 819 meeting, Persistence and Application of Entomopathogenic Nematodes, May 1995, Todi, Italy).

lation additives or tank adjuvants are applied in drops not containing nematodes. As application volumes increase, this wasted proportion of carrier liquid will also increase.

Fungal spores.

The example used is AQ_{10}(R), applied at 125×10^9 spores/ha (25 g/ha @ 5×10^9 spores/g), using three sprayers: two hydraulic flat fans (Hardi 8020-10 and -30) spraying water, a silicone surfactant, and a polymer-based drift retardant; a generalised airblast sprayer, spraying optimally (small drops, narrow drop size range) to badly (larger drops, wider drop size range); and a spinning disc (Micron Ulva+), maximum rotational speed, three throughputs, spraying a water-based formulation. The data is summarised in Table 1 and explanatory footnotes. Hydraulic nozzle and spinning disc drop spectra were obtained using an Aerometrics phase-Doppler particle analyser (for methodology, see Chapple and Hall, 1993). Data for the airblast sprayer was not easily available, and was modelled on distributions and particle size medians (pers. comm. R. Bateman, IIBC, Silwood Park, Ascot, UK).

For the purposes of this paper, AQ10 has advantages in that only one viable spore need be deposited close to a fungal mycelium for a toxic effect. Having two or more spores invade the same piece of mycelium is a waste of effort. Often this is not the case with other biological control agents: many myco-insecticides need multiple spores to obtain a lethal effect or to kill the insect within an acceptable period of time. However, irrespective of the number of spores required for an effect, the same general conclusions apply: all that has changed is the size of the "sample" (ie. drop size) required to contain an effective dose of the BCA.

With reference to Table 1, the hydraulic nozzle data demonstrate the effects of using two different spray adjuvants. From the water data, there are roughly the same number of spores/ha as drops/ha produced, but not all drops contain spores because of the wide range of drop sizes (cf. drift retardant VMD and NMD, Table 1: see also footnote **c**). At best, 46.6% of the spray contains no spores and wastes 0.3% of the applied surfactants / formulation components (ie. 8020-10 nozzle, polymer). However, the efficiency of the distribution is very low (2.7%). If a silicone surfactant is used, this appears to increase the % drops containing no spores (to 72.2%), to increase the wasted spray volume (to 16.9% with no

spores), but vastly increases the efficiency of the spray cloud overall (from 2.25% to 51.5%) This increase in efficiency is less pronounced with increasing l/ha and orifice size, although it should be noted that changing nozzle to the 8020-30 nozzle and 560 l/ha produces more drops containing no spores (ie. missed opportunities for pest control).

Table 2. Minimum drop size containing lethal ('n') number of spores, % drops and % volume containing sublethal numbers of spores (or none) for 8020-10 nozzle: hydraulic nozzle, no adjuvants, and spinning disc, water and oil based formulations.

No. ('n') of spores required for lethal or acceptable effect		Minimum drop size containing 'n' spores:	% drops with sub-lethal or no spores:	% volume with sublethal or no spores:
Hardi	1 spore	111.3 μm	64.0%	9.8%
8020-10	2 spores	140.4 μm	74.2%	17.5%
91 l/ha	5 spores	190.6 μm	85.6%	35.7%
	10 spores	240.2 μm	91.8%	54.9%
Hardi	1 spore	196.9 μm	86.6%	38.2%
8020-10	2 spores	248.0 μm	92.5%	57.7%
500 l/ha	5 spores	336.7 μm	96.9%	79.1%
	10 spores	424.3 μm	98.4%	88.6%
Spinning	1 spore	39.9 μm	14.8%	2.2%
disc	2 spores	50.3 μm	29.3%	6.9%
4.2 l/ha	5 spores	68.3 μm	53.8%	23.3%
(ULV:water)	10 spores	86.0 μm	73.4%	48.6%
Spinning	1 spore	28.4 μm	2.5%	0.2%
disc	2 spores	35.8 μm	9.5%	1.1%
1.5 l/ha	5 spores	48.5 μm	27.0%	6.0%
(ULV: oil)	10 spores	61.2 μm	44.5%	15.4%

The airblast data demonstrate three points. **1.** Although the number of drops containing no spores seems unconscionably high (as is the % volume), delivery efficiency approaches 100%. There are far more drops/ha than spores, and the drop size range is narrow relative to the hydraulic nozzle (Fig. 2): the spores are distributed evenly through the spray cloud. **2.** The increase in efficiency is obtained at the cost of wasting >95% of the spray volume and its included adjuvants. Many fungal-based BCAs are applied with adjuvants or formulated with enhancement agents (eg. humectants, anti-evaporants, oils). Unless these are tightly bound to the BCA and remain with the BCA upon atomisation, then the vast majority of the additives are being applied to the canopy anywhere but with the BCA. **3.** Increasing application volume from 250 l/ha to 800 l/ha appears to confer no advantage.

The spinning disc data demonstrates a compromise between the above two application systems (see also Bateman R., these proceedings). Although application volumes are very low (typically < 5 l/ha), efficiency approaches or betters that of the hydraulic nozzles while the wastage of formulation components and adjuvants is kept relatively low (1.5% to 7.4% in this example). The missed opportunities to deliver the BCA to the target are lower than for either the other two types of sprayer.

Table 2 indicates the change in % number of drops and % applied spray volume containing sublethal numbers of spores (or no spores) for a given lethal spore dose (ie. 1, 2, 5, and 10 spores), and the minimum drop size that will contain that number of spores. The data from Table 2 demonstrates two points. **1.** As the number of spores required for a lethal or timely effect increases, the wasted proportion of the spray - that containing no

spores or "sublethal" numbers of spores - increases markedly. **2.** Not only does the drop size required to obtain >1 spore per drop increase with increasing spore numbers/drop and application volume, but the size of the drop well exceeds that for efficient dispersal through a canopy (cf. airblast sprayer data, Table 1) or impaction on targets the size of insects. Clearly, if more than 1 spore is required for a lethal / timely effect, then the insect is picking up spores from a number of smaller drops as separate events, rather than being exposed to just one deposit cpntaining >1 spore as a single event. For sessile pests - including weeds, where often a number of spores are necessary at an infection site - obtaining the relevant number of spores per site is entirely dependent on the delivery system and/or redistribution of the deposit.

Conclusions.

The particulate nature of biological control agents requires a different approach to application and formulation compared to more the more traditional approach of dissolved or emulsified agrochemicals. High application volumes do not necessarily translate to better "cover", as it is the distribution of the BCA particles through the canopy that is of interest rather than the distribution of the carrier liquid. Use of adjuvants can appreciably change the efficiency of delivery: however, the soluble or emulsified nature of most formulation components means that their delivery to the target may not coincide with the delivery of the BCA, leading to excessive and costly waste. Delivery system and operating parameters (eg. droplet spectra, application volume) must be matched to the BCA but also to the target (eg. foliage, sedentary or in-flight insects), and formulation must be matched to the BCA, the target, and the application system.

References

Chapple A.C. and Hall F.R. 1993 "A description of the droplet spectra produced by a flat fan." *Atomization and Sprays* (Journal for the Institute for Liquid Atomisation and Spray Systems) **3:**(4) 477-488.

Chapple A.C., Downer R.A., and Hall F.R. 1992 "The effect of spray adjuvants on swath patterns and droplet spectra." *Crop Protection*. **12**:579-590.

Cooke B.K., Hislop E.C., Herrington P.J., Western N.M., Jones K.G., Woodley S.E., Chapple A.C., 1986 "The physical, chemical, and biological appraisal of alternative spray techniques in cereals." *Crop Protection* **5:** 155-164.

Graham-Bryce I.J. (1983) "Pesticide Research for the Improvement of Human Welfare." In: *Pesticide Chemistry: Human Welfare and the Environment*. Eds Miyamoto J and Kearney P.C. Volume 1. Synthesis and Structure-Activity Relationships. Vol. 1 Eds. Doyle P. and Fujita T. Pergamon Press.

Krueger H.R. and Reichard D.L. 1985 "Effect of Formulation and Pressure on Spray Distribution Across the Swath with Hydraulic Nozzles." *Pesticide Formulations and Applications Systems: Fourth Symposium, ASTM STP 875*, T.M. Kaneko and D.L. Spicer, Eds., American Society for Testing and Materials, Philadelphia, USA pp. 113-121.

LeFebvre A.H. 1989 "Atomization and Sprays" Hemisphere Pub. Co. NY., London 421 pp.

Appendix

To calculate the amount of the application volume atomised without nematodes (ie. the percent of formulation components or spray tank adjuvants not associated with nematodes):

$$\frac{AppVol - [NemHa \times minDS]}{AppVol} \cdot 100$$

where: AppVol= application volume in m^3 (eg. 242 l/ha = 0.242 m^3/ha); NemHa = number of nematodes per hectare (eg. 500000/m^2 = 5x10^9/ha); and minDS = volume (in m^3) of minimum drop size necessary to contain one nematode.

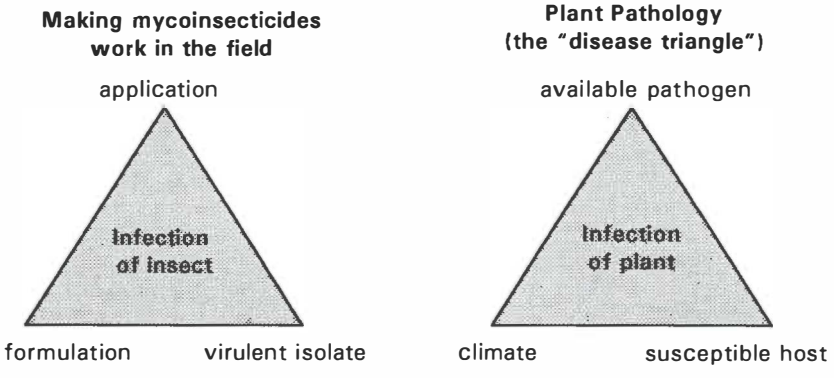
FORMULATION STRATEGIES APPROPRIATE IN THE ULTRA-LOW VOLUME APPLICATION OF BIOLOGICAL INSECTICIDES

Roy Bateman
International Institute of Biological Control (IIBC), *Ascot, Berkshire, SL5 7TA, UK.*

Abstract
LUBILOSA* and other projects have been able to show field infection of acridid pests with Deuteromycete fungus conidia, accompanied at times with significant reductions in pest population. Important contributory factors have included: the use of highly virulent isolates, vegetable or paraffinic oil formulations and controlled droplet application. A range of strategies are under evaluation for using 'Green Muscle®' (containing the conidia of an acridid isolate of *Metarhizium*) with most of the ultra low volume(ULV) application equipment used in locust control. It is easiest to store the conidia dry as a "technical concentrate" (TC) in order to maintain viability although a long shelf life with ULV suspension (SUs) is also possible.

The most widely used method to date has been to suspend the TC in non-toxic oil mixtures of a specified viscosity: either a short time (<3 months) before - or immediately prior to - application in the field. The latter most commonly takes place in trials where large quantities (>50 l) of SU must be prepared, but mixing can be difficult and there is always a risk with inhalation of very small (<10μm) particles. The preparation of oil miscible concentrates (OFs) is a possible solution, but rigorous quality control standards will be required in their preparation. Like their water-based (SC) equivalents, OFs typically exhibit strong non-Newtonian fluid flow characteristics. These are significant when diluting or directly spraying at ULV rates of application, and the implications are discussed here.

Fig. 1. An analogy with a concept in Plant Pathology



"LUtte Biologique contre les LOcustes et SAuteriaux" is a collaborative research programme being executed by IIBC, The International Institute of Tropical Agriculture, Cotonou, Benin and The AGRHYMET Centre, Niamey, Niger. LUBILOSA is funded by: the Canadian International Development Agency (CIDA), the Directorate General for Development Cooperation of the Netherlands (DGIS), the Swiss Development Corporation (SDC) and the Overseas Development Administration of the UK (ODA).

INTRODUCTION

I believe that success in the field (Bateman *et al*, 1994, Lomer *et al, 1996*) results from a combination of virulent isolate, appropriate formulation and application (*Fig. 1*). Unfortunately the latter is neglected by many insect pathologists.

i. **Isolates:** LUBILOSA has now identified 33 strains of "*Metarhizium flavoviride*", isolated from acridid hosts, that appear to belong to a highly virulent, genetically similar, homologous group (tentatively named "var acridum"). This group was most virulent to *Schistocerca gregaria* under the conditions of a standard laboratory assay (Bateman, Carey, Batt, Prior, Abraham, Moore, Jenkins & Fenlon, submitted), and includes IMI 330189, our "standard strain" from Niger used in most tests to date.

ii. **Formulation:** Oil based formulations of *Metarhizium* are more virulent than suspensions in water against a Curculionid (Prior *et al.*,1988) and *S. gregaria*, (Bateman *et al.*,1993), especially at low humidity. 'Green Muscle®' is the name given to a group of oil-based or oil miscible products containing *Metarahizium* conidia for use with ultra low volume (ULV) equipment.

iii. **Application:** ULV is the conventional means of applying pesticides to Orthopteran pests such as locusts (Symmons, 1992). Unlike many other application systems (A. Chapple, these proceedings), the high concentration of conidia typically used in ULV suspension (SU) formulations ($>10^{12}$ conidia/l) ensures that virtually all droplets contain viable spores (Bateman, 1994). These are typically in the orders of 100-10000 conidia per droplet in the optimal 40-120μm range (FAO, 1992), thus ensuring that each droplet is potentially lethal if it directly hits the target. In practice 10-60% of the grasshoppers or locusts may not be directly hit, so good coverage on foliage to maximise secondary pick-up is important. In this particular application system we have been able to benefit from the efficiency accompanying controlled droplet application.

MASS PRODUCTION AND FORMULATION

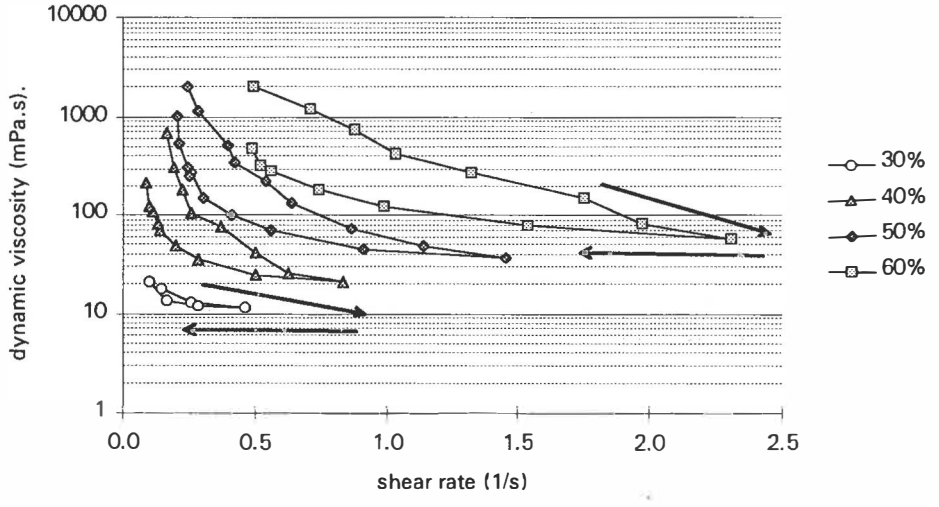
LUBILOSA uses a two phase system to produce aerial conidia with lipophilic cell walls needed to prepare oil-based formulations. Cooked grain is inoculated with a liquid mycelial concentrate, produced in shake flasks or a fermentor, and then allowed to conidiate in plastic bowls for approximately 10 days. The basic product is a technical concentrate powder (TC) consisting of >99% pure spores of IMI 330189, with a moisture content of <6%. Large particles in the TC cause blockages in ULV equipment and reduce the effective concentration of the ULV suspension. A low cost (<£5000) prototype fluid bed device is under development that separates TC from conidiated grain. It produces a high quality spore powder (with practically no particles >30 μm) by a mechanical process that minimises operator exposure to dust. The two stage process contrasts with existing commercial mycoinsecticide products (e.g. PFR®, Conidia®) which are based on fermentation techniques only (Jenkins and Goettel,1996); the resulting formulations are water miscible.

Currently, the dry spores are mixed with the spraying diluents at the time of application. Dry powders can be stored for a long time, but are difficult to handle in the field, and as with any dusty material, there is a potential inhalation problem. It is therefore desirable to develop innocuous formulations which are easy to pour and dilute in the field (Seaman, 1990). As with chemical formulations, "flowable" concentrates are an obvious technical solution. Oil miscible flowable concentrates ('OF' formulations) are the oil-miscible equivalents of the more familiar aqueous suspension concentrates (SC) and a feasibility study is currently being conducted. A well divided spore powder is essential in the development of an OF formulation and a shelf stability of 6 months will be required for the final product.

USE OF NON-NEWTONIAN FORMULATIONS WITH HAND-HELD ULV SPRAYERS

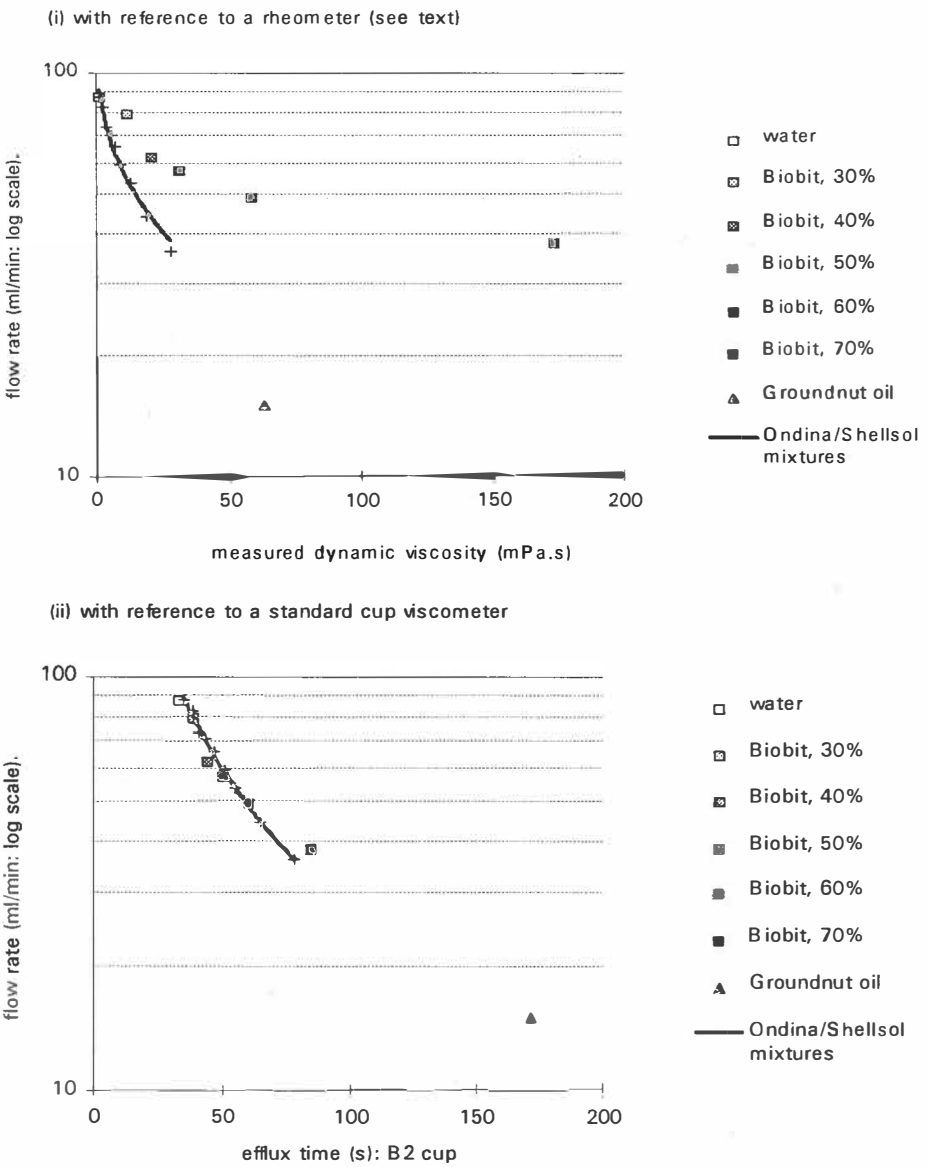
Water miscible biopesticide formulations such as wettable powders (WP) and water dispersible granules (WG) can be simply mixed with small quantities of water and sprayed with rotary atomisers (Bateman, 1994). However practical difficulties can arise when attempting to calibrate and spray undiluted "flowable" formulations such as SCs and OFs. The formulation must pass through narrow restrictor orifice, although the flow of viscous liquids may also be governed by the air bleed mechanism in hand held sprayers. Rheometers such as the Brookfield device and the Haake 'Rotovisco' are used to describe the dynamic viscosity at different shear rates, of "flowable" formulations (e.g. Sundaram & Retnakaran, 1987). *Fig. 2* shows rheometrical measurements taken with the Brookfield device using a four dilutions of 'Biobit XL®' (a commercial SC formulation of *Bacillus thuringiensis*) as an example.

Fig. 2. Sequential viscosity measurements of 4 concentrations of 'Biobit' in water, taken with a Brookfield DVII+ (LV1 spindle)



All dilutions are pseudoplastic (thinning with increased shear) and thixotropic (there is a lowering of viscosity with time that a shear force has been applied, which gradually rises on return to rest). What are the implications of this when using in a simple ULV sprayer mechanism (which is effectively a type of "viscometer")?

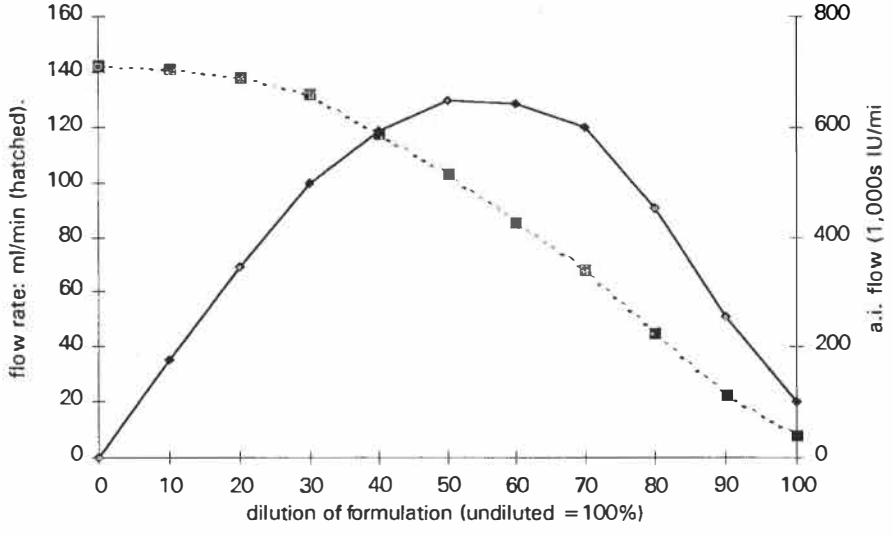
Fig. 3. Relationship between viscosity measurements and flow rates through a spinning disc sprayer ('Ulva+' fitted with a red restrictor, 20°C)



Walters (1980) has reviewed the various means of measuring viscosity and points out that different instruments **practically never produce comparable data** since the nature and speed of liquid flow is different in each; this is especially problematic with non-Newtonian fluids. The 'B' series of flow cups (British standard 1733 (1955) superseded by BS3900) are the possibly the cheapest, most convenient and robust method of assessing viscosity; measurements are expressed as an emptying (efflux) time at a given temperature. *Figure 3* shows that the use of simple cup viscometers may be a more reliable way of predicting the flow of non-Newtonian formulations in spinning disc sprayers than more sophisticated rheometers. In this case the Brookfield device reading at the highest shear rate (and therefore the lowest apparent viscosity) was used; however adapters are available that enable measurements at higher shear rates, that would have given results that were more compatible with the Newtonian fluids tested (Water, Ondina EL /Shellsol T mixtures in a range of ratios).

Figure 4 shows the flow rates of serial dilutions of 'Biobit XL' SC through an 'Ulva+' fitted with a black restrictor. The flow rates for mixtures containing up to 30% SC are little different than water, but then decrease with the increase in viscosity accompanying higher concentration. The resulting curve for flow of active ingredient shows a peak at approximately 50% dilution, although there is little change over the 40-70% range; in practical terms only the flow rate (affecting the volume application rate, coverage and work rate) would change.

Fig. 4. Flow characteristics of 'Biobit' through an 'Ulva+' (see text)



In conclusion: careful calibration is necessary with "flowable" concentrates. A range of formulation options are available for use with ULV sprayers; these must be reliable products, for which the method of mass production spore separation can be crucial.

References

BATEMAN, R.P. 1994 Physical properties and atomisation of ULV formulations of myco-insecticides. In "Microbial Control of Pests" Ed. P.H. Smits. *IOBC WPRS Bulletin*, **17 (3)**, 189-192.

BATEMAN, R.P. , 1996 Methods of application of microbial pesticide formulations for the control of grasshoppers and locusts. Review paper accepted by the *Memoirs of the Entomological Society of Canada*.

BATEMAN, R.P., BATT, D., CAREY, M., DOURO-KPINDOU, O.K., GODONOU, I., JENKINS, N.E., KOOYMAN, C., LOMER, C.J., 1994 Progress with the development of Metarhizium flavoviride for control of locusts and grasshoppers. In: "Microbial Control of Pests" Ed. P.H. Smits. *IOBC WPRS bulletin*, **17 (3)**, 222-225.

BATEMAN, R., CAREY, M., MOORE, D., PRIOR, C. 1993 The enhanced infectivity of M. flavoviride in oil formulations to desert locusts at low humidities. *Annals of Applied Biology*, **122**, 145-152.

FOOD AND AGRICULTURE ORGANISATION (FAO) 1992 Controlling desert locusts. Food and Agriculture Organisation Booklet, 64 pages.

JENKINS, N.E. AND GOETTEL, M. , 1996 Methods for mass production of microbial control agents of grasshoppers and locusts. Review paper accepted by the *Memoirs of the Entomological Society of Canada*.

LOMER, C.J., PRIOR, C. AND KOOYMAN, C. , 1996 Development of *Metarhizium spp.* for the control of grasshoppers and locusts. Review paper accepted by the *Memoirs of the Entomological Society of Canada*.

PRIOR, C., JOLLANDS, P. & LE PATOUREL, G. 1988 Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina; Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). *Journal of Invertebrate Pathology*, **52**, 66-72.

SEAMAN, D., 1990 Trends in the formulation of pesticides - an overview. *Pesticide Science*, **29**, 437-449.

SUNDARAM, A. AND RETNAKARAN, A. 1987 Influence of formulation properties on droplet size spectra and ground deposits of aerially-applied pesticides. *Pestic. Sci.*, **20**, 241-257.

SYMMONS, P. 1992. Strategies to combat the desert locust. *Crop Protection*, 11, 206-211.

WALTERS, K. 1980 Rheometry: Industrial Applications. Research Studies Press (J. Wiley & Sons).

COMBINED USAGE OF FUNGAL AND BACTERIAL BIOLOGICAL PREPARATIONS IN INTEGRATED PROTECTION OF THE POTATO

M.P.Lesovoj, S.V.Goral
Institute of Plant Protection (Ukr.Akad.Agr.Sci), Ukraine, Kiev

Abstract. It has been studied the effect of various concentrations of such biopreparations as bacterial bitoxibacillin, novodor, ecotec-bio and fungal boverin as well as its mixtures with first ones on Colorado potato beetle larvae with noting the damage (weight of eaten potato leaves) and mortality dynamics. The third instar larvae infected with entoomopathogen bacteria strains (as the bases of novodor and bitoxibacillin) appeared to retard feeding long before death. The complex usage of bacterial and fungal preparations, i.e. novodor with boverin, bitoxibacillin with boverin, suppressed larval feeding to a far greater extent than if being used alone. In so doing it required 6 and 4 times less rate of treatment to reduce feeding to the level of alone usage of above preparations. Bacterial preparation ecotec-bio showed weak activity with regard to larvae and its mixture with boverin was not only ineffective but suppressed the action of last one.

Three years of testing, carried out with noting the calculated effective rate of treatment of biopreparations and their mixtures, confirmed their effectiveness.

In Ukraine, areas with the potato of household gardens exceed that ones of state gardens very much. Therefore it's up to date problem to limit the use of chemical pesticides by using biological preparations. Ukrainian researchers are looking for fungal antagonists to *Phitiphtora infestans*; the results of laboratory and field tests are hopeful.

Colorado potato beetle *Leptinotarsa desemlinaeta Say*. is commonly widespread in Ukraine. So its control is required to grow the potato.

First of all biopreparations made on the base of cristalloforming bacteria *Bacillus thuringiensis* reduce the feeding of infected insects since cristalls of endogenous toxin in the cells of the above bacteria exert the paralyse effect to pests gut. However different serotypes of this group of bacteria act not in the same way (Лапша Н., Горал В.,1985, 1990).

The aims of present research were following:

to evaluate quantitatively the degree of suppression of Colorado potato beetle feeding after treatment with bacterial preparations bitoxibacillin, novodor, and ecotec-bio (which made on the base of cristalloforming bacteria);

to investigate the efficacy of the mixtures of above preparations with fungal preparation boverin;

to optimize the rate of treatment of biopreparations and their mixtures with usage of such parametres as larval mortality and ones harmfulness.

Materials and methods. The experiments were conducted in the laboratory. The third instar larvae were rearing in cages (10 individuals per cage) at 23-25°C. Larvae were once fed with potato leaves sprayed by biopreparations bitoxibacillin, novodor, ecotec-bio and boverin in concentrations of 0,0313; 0,0625; 0,125; 0,25; 0,5; 1,0% and by mixtures of bacterial and fungal preparations in the same concentrations. During 10 days the larval mortality and their damage (weight of eaten potato leaves) were checked daily.

To describe the mortality dynamics, the level of larval harmfulness and optimal rate of treatment of biopreparations such indexes as: time within 90% of larvae dead (td_{90}); time within damage declined to 10% (tdm_{10}); damage below 10% (D_{10}); calculated effective rate of treatmennt of biopreparations (ERT) were input. Account of effective rate of treatment of biopreparations carried out by known methods. The tests results were processed statistically.

Results. Data of larval mortality, weight of damaged leaves and afteraction of preparations are shown in Table 1. From those data one can see that the more infectious pressure the faster larval death and the fewer their voracity, especially if to use mixtures of boverin with bitoxibacillin and novodor. As for ecotec-bio, this preparation almost didn't lead to significant mortality and its mixture with boverin even retarded the action of last one.

The comparison of efficacy of novodor, bitoxibacillin and their mixtures with boverin showed that td_{90} and ammount of damaged leaves were approximatelly 30% and 50% (accordingly) less for mixtures than for preparations alone. Also, mixtures of preparations exhibited afteraction effect (Table 1).

Table 1 presents the dynamic of feeding of infected larvae and calculated dimensions of tdm_{10}. It's shown that tdm_{10} for mixture of boverin with novodor was 30% less than if to use these preparations alone; there was no significant differs in tdm_{10} between bitoxibacillin, boverin and their mixture.

The mixture of ecotec-bio with boverin had some promote effect on intensity of larval feeding.

Table 2 presents the calculations of effective rate of treatment (ERT) of biopreparations and their mixtures which were carried out with usage of larval mortality (LD_{90}) and their damage (D_{10}). Effective rate of treatment for boverin calculated by LD_{90} and D_{10} got equal 4,5 kg/ha and 4,0 kg/ha accordingly; for novodor - 4,3 and 1,7 kg/ha; and for their mixture - 1,13÷1,13 and 0,7÷0,7 kg/ha.

Three years of testing, carried out with noting the calculated effective rate of treatment of biopreparations and their mixtures, confirmed their effectiveness. In usage of mixtures of bitoxibacillin and boverin in rates of treatment two times less than optimal, protection of crop was at the level of chemical preparations (phosalon, decis) usage.

Conclusion

The above data allowed to conclude that larvae of Colorado potato beetle infected with entomopathogen bacteria strains (as the bases of novodor and bitoxibacillin) stopped feeding long before death.

Usage of bacterial-fungal mixtures - novodor with boverin and bitoxibacillin with boverin - suppressed feeding of Colorado potato beetle larvae significantly more than if the same preparations were used alone. In so doing it required 6 and 4 times less expenditures to reduce damage to the level of alone usage of above preparations. Also, the times between cessation of harmful action of larvae and their death were significantly different.

Therefore it should use the mixtures of boverin with novodor and boverin with bitoxibacillin not only to reach biological efficacy at determined phases of potato growth which are most susceptible to injury. So different types of preparations act in different ways: bacterial - through gut, fungal - through integument, and besides these preparations are capable to accumulate into population.

Usage of indexes of feeding delay allows to optimize the expenditures of biopreparations in every spesific situation.

Bacterial preparation ecotec-bio showed weak activity with regard to larvae of Colorado potato beetle. Consequently its mixture with boverin was not only ineffective but suppressed the action of last one.

Three years of testing, carried out with noting the calculated effective rate of treatment of biopreparations and their mixtures, confirmed their effectiveness.

References

Горань В.М., Лапша Н.В. Эффективность битоксибацилина в борьбе с колорадским жуком на Украине.//Тезисы докладов Республиканской научно-практической конференции.-Кишинев:Агропромиздат МССР,1985.-С.63-64.

Лапша Н.В., Горань В.М. Определение нормы расхода и биологической активности битоксибацилдина на колорадского жука. Методические указания. М.:ВО "Агропромиздат",1990. -14с.

Table 2. Calculation of effective expenditures of biopreparations and their mixtures against Colorado potato beetle

Dimension in infections pressure, % for preparations in usage		Bove-rin	Novo-dor	BTB	Boverin in mixture with	
alone	in mixture				novodor	BTB
a. Larval mortality (% with correction to mortality in control) at 6-th day after treatment						
0.0625	0.0313+0.0313	11.5	55.8	55.8	43.0	55.8
0.125	0.0625+0.0625	32.8	61.4	55.8	64.9	66.0
0.25	0.125+0.125	53.3	72.8	59.2	81.8	86.4
0.5	0.25+0.25	72.8	79.6	86.4	96.6	99.9
1.0	0.5+0.5	93.2	94.6	96.6	99.9	99.9
LD_{90}*, %		0.89	0.87	0.82	0.23+0.23	0.2+0.2
ERT**, kg/ha		4.5	4.3	4.1	1.13+1.13	1.0+1.0
b. Decline of feeding intensity (% of control) in infected larvae						
0.0625	0.0313+0.0313	9.7	62.2	44.8	61.1	65.5
0.125	0.0625+0.0625	44.8	86.2	50.0	89.7	89.7
0.25	0.125+0.125	62.1	93.1	55.6	100	86.2
0.5	0.25+0.25	72.4	96.6	93.1	100	100
1.0	0.5+0.5	96.6	96.6	100	100	100
D_{10}***, %		0.78	0.33	0.65	0.13+0.13	0.15+0.15
ERT**, kg/ha		4.0	1.7	3.3	0.7+0.7	0.8+0.8

* LD_{90} - larval mortality
** ERT - effective rate of treatment
*** D_{10} -damage below 10%

Table 1. The effect of boverin, novodor, bitoxibacillin, ecotec-bio and their mixtures on Colorado potato beetle larvae

Variant of experiment	Concentration, %	Dynamic of larval mortality (%) per day of experiment			Adult emergence %	td_{90}, days	Weight of eaten potato leaves, g/10 larvae	td_{10}, days
		2-nd	6-th	10-th				
Control		0	2.0	8.2	74.0		3.53±0.012	
Boverin	0.25	5.8	54.4	100	0	8.7±0.14	1.95±0.013	8.4±0.16
	0.5	4.4	73.3	100	0	8.3±0.18	1.61±0.059	8.6±0.06
	1.0	6.2	93.3	100	0	6.6±0.27	0.61±0.059	6.5±0.46
Novodor	0.25	2.2	73.3	76.7	13.3	9.9±0.50	0.43±0.016	8.3±0.13
	0.5	2.2	80.0	86.4	6.7	9.1±0.44	0.77±0.026	7.0±0.16
	1.0	6.7	86.7	93.3	0	7.8±0.62	0.51±0.037	6.8±0.22
Boverin + Novodor	0.125+0.125	4.6	82.2	100	0	7.5±0.27	0.54±0.021	4.9±0.08
	0.25+0.25	6.7	96.7	100	0	6.5±0.16	0.44±0.016	4.6±0.01
	0.5+0.5	6.7	100		0	3.9±0.01	0.35±0.009	4.2±0.01
BTB	0.25	10.0	56.7	70.0	16.7	11.3±0.19	2.09±0.058	10.6±0.56
	0.5	0	86.7	96.7	3.3	6.5±0.38	1.08±0.038	6.0±0.15
	1.0	13.3	96.7	100	0	5.3±0.24	0.54±0.006	4.6±0.33
Boverin + BTB	0.125+0.125	3.3	86.7	100	0	7.2±0.35	1.18±0.015	6.1±0.13
	0.25+0.25	3.3	100		0	5.2±0.01	0.76±0.019	5.0±0.13
	0.5+0.5	6.7	100		0	3.8±0.03	0.53±0.013	4.7±0.11
Ecotec-bio	0.25	1.1	14.4	18.9	80.0	39.3±0.18	3.77±0.026	69.9±0.51
	0.5	2.2	22.2	27.8	66.7	27.4±0.21	2.96±0.026	20.2±0.26
	1.0	3.3	36.7	50.0	46.7	17.8±0.27	2.83±0.052	18.3±0.20
Ecotec-bio + Boverin	0.125+0.125	5.5	37.8	94.4	3.3	9.4±0.14	1.89±0.020	11.1±0.03
	0.25+0.25	5.5	52.2	97.8	0	8.9±0.13	1.64±0.031	6.1±0.15
	0.5+0.5	0	66.7	100	0	8.4±0.22	2.21±0.053	7.2±0.19

TOXICOLOGICAL EVALUATION OF THE BIOLOGICAL PREPARATIONS (BP)

R.B. BOROVIK, N.R.DYADISHCHEV, S.P.RYBALKIN, K.G.SOLOVIEV & N.M.ONATZKY

Centre of Toxicology and Hygienic Regulation of Biopreparations (CT HRB)
of Ministry of Health and Medical Industry of RF, 102 Lenin str.,
Serpukhov, 142283, Russia

Summary

Three problems are considered in the present report:
1) interpretation of the notion "biological preparation";
2) selection of the model which is adequate to a human organism;
3) evaluation of the aerogenic way of BP administration.

Results and discussion

1

Discussions are being conducted (1,2,3) about the term of "biological preparation" (BP). We support "extended interpretation" of this term, including in it the following groups of substances:
1. Native products of biological origin created on the base of cells of macro and microorganisms or on the base of products of their synthesis in vivo.
2. In vitro synthesized products corresponding by the structure to the natural ones.
3. Products of the 1st and 2nd groups subjected to destructural changes under the influence of chemical, physical and biological factors.
4. All the "pollutants of biological nature" from one-piece cells to subcellular structures which have the ability to multiply in the conditions of the natural environment.

2

The problem of choice of the adequate model arises with evaluation of the impact of BP on one's organism. Laboratory animals, whose responce reaction on BP is the closest to a man, are traditionally used. There is a point of view that the refusal at all or at least the reduction of number of experoments with animals is expedient (4,5). Rabbits, quinea-pigs, white rats and mice are used most often. Monkeys are taken more rarely, when the the specific conditions of the experiment require it. Well-known methods of

injections such as inderskin, intravenous ets, are not so important for this discussion. At the same time the biggest part of the plant protection means are produced as a powder in Russia. Thus there is a process of BP's being inhalated into the man's respiratory tract during both the production and the application.
The experiments showed that only monkeys and white rats can be the adequate models for the aerogenic investigation. Men and these two kinds of animals have several common morphophysiological features (6). These features are:
1. Relatively small length of respiratory tructs of primates and white rats comparing to quinea-pigs and rabbits.
2. Both men' and white rats' lymph nodles are situated in the walls of bronchial tubes even emerging into mucous memrane.
3. When the pulmonary veins branch out, peculiar "cases" are forming from muscular fibres. Both men and white rats have strengthened muscles in the system of pulmonary veins, moreover cross-striped muscle fibres are used for it. At the same time muscles of pulmonary veins of rabbits and quinea pigs are strengtgened by smooth muscles. The structure of tructs passing air into the lungs is almost analogous for both men and monkeys. Among small laboratory animals, white rats are the closest to man by the structure of upper respiratory tructs. These anatomical features are the reason for the fact that even large particles of BP (but not bigger than 1-5 micrometres) are able to get into the lungs aerogenically.

3

There are two methods of simulating of BP's impact on animals and man in the laboratory conditions:
1. Aerogenical promotor in chamber installations.
2. Intranasal and intratracheal injection.
The aerogenical primer method is the most adequate model of real impact caused by the pollutants of aerial environment on one's health, in spite of all the technical difficulties, big expenditure of labour and expenses. Intranasal and intratracheal methods cannot simulate such an impact because of several reasons. The preparation in a dry state should be injected in the respiratory tructs in the same state. During the intranasal and intratracheal injection the preparation is rehydrated in the liquid state at first. Then, in the liquid state, it is injected in the respiratory tructs. Besides, in this case the preparation is getting into the organism of the animal not only through respiratory tructs but also through their damaged areas right into the blood. The applying of intranasal and intratracheal methods of injecting of the tested preparation into the respiratory tructs is admissible only during the preliminary testing of the substances. Toxicological evaluation of the finished products of Plant Protection Means applying impact on the laboratory animals should be conducted using only special

chamber devices with strict adherence of sanitary standards and rules.

The stage of preparating of the dry substance for testing is very important. The particles in the dry biological preparations may widely fluctuate in size. The biggest part of them are not able to fly in the aerosol of the atmosphere or primer chamber.

It is certain that the testing of the dry final product in the aerosol primer chamber without any preliminary preparations does not give effective results. Because of this it is necessary to prepare the substance for converting into the aerosol of the primer chamber by separating of flying fraction (less than 100 micrometres).

The method of preparing consists of 4 stages:

1. Preliminary separation of the fraction less than 100 icrometres.
2. Mild "grinding" of the separated substance.
3. Mixing of all the parts of separated and grinded substance into one preparation.
4. Its physical and biological analysis.

The important and requiring attention factor is the impact of climatic conditions inside the primer chamber on the viability of microbe cells of the tested subctance. Considering the fact that biological Plant Protection Means can be produced both in liquid and dry state, climatic conditions (especially relative humidity) should be taken into acount while testing the preparation in the working zone of chamber. Microbiological practice says that bacteriological cells of the dry final product are most stable in the aerosol with low relative humidity (less than 50 %). On the contrary, cells of the liquid final product are stable in the aerosol with high (more than 80 %) relative humidity. Hence, the testing of the preparation using the method of aerogenical promotor (priming) should be conducted either with low relative humidity (not more than 50-60 %) if the preparation is dry or with high relative humidity (not less than 70 %) if the preparation is liquid. Thus only the right choice of the model for the testing of and its necessary cheking in the chamber conditions will give us objective knowledge about the impact of BP on the respiratory truct of a man.

Literature cited

1.Alekseeva O.G.//Tox.vest.-1994.-n.6.-p.2.
2.Sergeyuk N.P., Pohudey U.I.//Tox.vest.-1994.-n.6.-p.5.
3.Krugliy stol//Tox.vest.-1994.-n.6.-p.30.
4.Mezhd.konf.po him.bezopasnosti//Tox.vest.-1994.-n.3.-p.42.
5.Rybalkin S.P. with co-authors//Tox.vest.-1994.-n.6.-p.11.
6.Kovalevskiy G.V.//Docl.AN SSSR.-1966.-T.168.-n.5.-p.1214.

THEORETICAL AND PRACTICAL ASPECTS OF MICROBIOLOGICAL PRODUCTS USED IN BELARUS

Vilor Samersov, Luidmila Prischepa

Biological Methods Department Belarusian Institute of Plant Protection p/o Priluki 223011 Minsk, Belarus

Summary

To improve the sanitary conditions of the ecological situation and to reduce the volumes of chemical insecticides use it is necessary to use biological products, having the unique biological background directed to maintain the dynamic balance and resistant equilibrium of *Arthropoda communites* in biocenosis. In the Republic of Belarus the research on the development of the microbiological method of agricultural crop protection against pests is organized in several direction, the among them is a search, the selection of local highly virulent strains as the basis for the production of biological preparations and the development of their effective use for the protection of fruit, vegetable, cereal and potato crops.

Introduction

The search of entomopathogenic bacteria, fungi, viruses has been carried out in Belarus since 1962 in the natural populations of fruit, vegetable pests, forest plantations and soil. While investigating the population of insects, the entomopathogenic microorganisms have been selected from the dead larvi, caterpillars, pupi of the orders Lepidoptera, Coleopicta, Dermaplera. Microbiological preparations get based on highly active entomopathogenic bacteria (Korol et al., 1984), fungi (Korol et al., 1982), viruses (korol et al., 1984) strains isolated in agrocenosis in Belarus are the effective components of contemporary integrated systems.

Materials and Methods

Isolation of entomopathogenic fungi, viruses, bacteria was accomplished based on Insect Pathology methods. Under laboratory conditions the populations of *Orgyin antiqua L., Galleria mellonella L.* and *Laspeyresia pomonella L.* are being reared to determine the allelic samples of the bioprepations prepared on the local strains of entomopathogenic microorganisms. Moreover, to select strains with a high biological activity the spectrum of ????? different systematic orders was studied. All the experiments were performed in 6 repetition taking 20-25 caterpillars in the repetition. To select the strains with a high biological activity, the taxonomic properties, productivity on cheap nutritive media are determined.

Results and Discussion

In the course of research only once the epizocty in the population of *Dolerus sp.* was noticed on barley, in other cases.

Table 1.

Lepidoptera	caterpillar	3	H_{4a-4b}
Hymenoptera	pupae pseudocaterpillar	2 1	H_1 H_{4a-4b}
Coleoptera	Imago larvae pupae	1 2 3	H_{10} H_{4a-4b} H_{3a-3b}
Coleoptera *Carabidae*	Imago	2	H_{10}
Coleoptera *Elatoridae*	Imago pupae	2 1	H_{4a-4b} H_{4a-4b}

Coleoptera Curculionidae	Imago	2	H_{4a-4b}

To select the strains with a high biological activity, the taxonomic properties, range of the entompathogenic action or the wide spectrum of pests from different systematic order, productivity on cheap nutritive media are determined. The developed system of selection allows to create a collection of the entomopathogenic of fungi *P. beauveria,* bacteria *P. bacillus thuringiensis, Baculovirus* (50 highly active local strains), to develop experimental and industrial technology of the product Boverin, the bacterial products Dendrolin and Coleptexin and the virus ones VIRIN-CGV, VIRIN-KSH, effective in the control of leaf-biting pests of cabbage, apple-tree, potato.

Based on our perennual research on mechanism of the biological product action on pests and the contemporary level of knowledge on the role of the biological agent in agrobiocenesis, the main stages of the technology of their use in the integrated system approach is to the efficiency evaluation or the microbiologically substantiated and the rational technology of their use in the integrated systems of agricultural crops protection (Korol et al., 1981, 1982, 1983, Samersov, 1988, 1994, Samersov et al., 1995).

Table 2. Stages of the technology of biopreparations use development in the integrated systems of Agricultural crop protection against pests.

Laboratory evaluation of the biological preparation	1. Lethal concentrations for pests, LC 50 2. Action on the efficiency of pests 3. Action on pests fecundity 4. Teralogenic and other effects 5. Compatibility with fungicides and other products
Field evaluation of the biological preparation	1. Optimal rates 2. Time and number of applications 3. Efficiency in pure form and in mixture with other means 4. Plant persistance
Production testing of the biological preparation	1. Protective and economic effect 2. Influence on the complex of useful insects

The technology of microbiological preparations use in the control of apple tree orchards, cabbage, potato pests recently the possibility of using the biological means on technical fodder and cereal crops is investigated. The use of biological preparation ensures 70-90% pest death and the replace of 1-3 chemical treatment, depending on the crop type and the pest number.

ACTUAL AND POTENTIAL MARKET FOR BIOLOGICAL CROP PROTECTION AGENTS IN POLAND

Andrzej Bednarek/1/, Wojciech Goszczyński/2/ and Anna Pawłowska /3/

/1/ Department of Zoology, Warsaw Agricultural University /SGGW/, Warsaw, Poland
/2/ Department of Applied Entomology, Warsaw Agricultural University /SGGW/
/3/ ROL-EKO Ltd, Horticultural Promotion Office, 02-958 Warsaw, Wiertnicza str. 1 G

In Poland there is a relatively high horticultural production in comparison to other European countries. A total vegetable yield in 1993 reached 6138 thousand tons, of which 4.2% came from crops grown under cover. In the same year the commodity value of horticultural production totalled ca. 100 Million ECU, i.e. 15.1% of a total agricultural value. Horticultural crops cover around 280 thousand Ha, where the production under cover constitutes 0.87%.

Integrated production methods, in particular integrated and biological crop protection, are an essential element of modem horticultural production. In Poland biological agents of plant protection have been used on crops under cover since the 1970s, however, a progress in this field took place after 1980. Activity of the Plant Protection Institute in Poznań gave rise to the production of entomophages in several large horticultural farms under state management (SLH). For example, entomophages */Phytoseiulus persimilis* and *Encarsia formosa/* reared in the SLH farm at Naramowice /Poznań/ had been applied within an area of 5 Ha. At the same time ERNA, a co-operative then private unit producing entomophages, had been set up for supplying horticultural farms in the south of Poland. According to the Plant Protection Institute, at that time 500 Ha of crops under cover had been subjected to biological methods.

In the early 1990s a number of SLH farms and and also small horticultural farms collapsed. At the same time SLH farm ceased to rear entomophagous species for their own use, while ERNA closed down their production due to the adverse tax policy. An essential factor which contributed to the end of entomophages production was the activity of large foreign companies supplying reliable, high quality products. Since 1991 a Dutch company, KOPPERT Biological Systems, has been operating in Poland together with its distributor, ROL-EKO Horticultural Promotion Bureau. In the following years other companies began to operate in Poland, i.e. BIOBEST /Belgium/ represented by BIO PARTNER /Poznań/, and BRINKMAN /Netherlands/ which set up its own company. Moreover, the horticultural farm at Owińska /Poznań/ undertook the commercial production of entomopathogenic nematodes. Recently in Poland there is a development of distribution /advisory network co-operating with suppliers of entomophages. At present, it is estimated that above 40 units within the country provide biological control products for crop protection. According to the Research Institute of Vegetable Crops, Skierniewice, in 1993 the biological method was applied in a total area of 480 Ha under cover.

For the past three years the Research Institute of Pomology and Floriculture, Skierniewice, has been co-operating with a group of above 570 commercial orchards, providing them with self-supplied entomophages, in order to implement an integrated method of apple production. Moreover, bacterial plant protection agents are being applied in a limited area of field vegetable crops and potatoes against the Colorado beetle and caterpillars, as well as against forest pests.

The present report attempts to assess the market of biological products for crop protection in Poland, with regard to prospects for its development. However, considering a large extend of this problem we are limited to look only at crops protection under cover.

Specificity of horticultural production requires to separate problems of pest control for particular crops and types of farms /Table 1/. Most of the productive area is covered by plastic tunnels, but only a minor part of them is heated and provided with drip irrigation. A private horticultural farm of an average size includes several tunnels of 250 m^2 each, where the production is based on a simplified technology during a shortened season. In some tunnels, particularly those heated in spring, there are two production cycles: one in spring with a cucumber crop, the second in summer with tomato. In such areas microclimatic conditions are highly unfavourable. Serious problems associated with plant protection, fungal diseases in particular, appear towards the end of the production period. So far, entomophages have been applied on a very limited scale /against whitefly and aphids/.

Table 1. Area of crops under cover and relevant pests.

Object	Total area of crops	Crop	Structure of crops	Major pests
Big farm < 1.5 Ha	225 Ha	Tomato	88%	whitefly, spider mite, leaf miners, sciarids
		Cucumber	10%	thrips, spider mine, whitefly, scarids
		Other vegetables	2%	spider mite, aphids
Small farm > 1.5 Ha	780 Ha	Tomato	60%	whitefly, spider mite, leaf miners
		Cucumber	30%	spider mite, whitefly
		Other vegetables	10%	aphids, spider mite
Plastic tunnels	1485 Ha	Tomato	50%	whitefly
		Cucumber	35%	whitefly, spider mite
		Other vegetables	15%	aphids, spider mite
	800 Ha	Ornamentals		spider mite, whiteflies, aphids, thrips, sciarids, aphids, sciarids, wingless weevils

Greenhouse farms not exceeding 1.5 Ha /av. 800m^2/ vary in their production standards and introduction of advanced technology. Modern units apply rockwool and drip irrigation, being rarely provided with microclimate regulation systems. In such greenhouse entomophagous species and bumble bees for pollination are being used. Tomatoes are grown an all year round cycle, although in most cases there are two production cycles: one in spring with cucumber, the second in autumn with tomato.

Autumn chrysanthemum crop is preceded by tomato grown in a spring cycle, similarly to a practice in tunnels.

SLH farms /from 1.5 Ha to above 30 Ha under glass/ have been partially privatised and now most of them are undergoing a modernisation process. Crop grows under qualified specialists include either tomato in a year cycle, or cucumber in the first cycle followed by tomato or cucumber again.

Market for entomophagous species analysed for the period of 1991-1995 turned out to be highly diversified, depending on a species applied and type of farm /Table 2/., based on the ROL-EKO data. There is a gradual significant increase of beneficial insects applied within smaller farms, apparently as a result of their availability from expanding distributing services, proved by a rising number of customers. It appears that tomato growers usually begin to apply biological systems with a programme of natural pollination, followed by the biological control of whitefly, then introducing other entomophages.

After an initial strong increase in numbers of entomophages applied within SLH farms, from 1993 there has been their stabilisation /*E. formosa*/ or significant decline /*Ph.persimilis*, *A. cucumeris*, *Orius sp.*/. It could be associated with decreasing pest populations on crops as a result of the intensive biological control conducted in previous years. Another factor is a sudden decline in the area of cucumbers grown in SLH farms, caused by low production profitability. Data presented in Table 2 should be adjusted with regard to an expected division of the market between competing suppliers of entomophages. In SLH farms the biological control of leaf miners and aphids with the use of predators and parasitoids is less intensive than in other farms, although these pests could be of great importance.

Table 2. Developing market of entomophages applied within greenhouse farms in Poland during 1991-1995, based on ROL-EKO data.

Entomophagous species	Farm type	Relative increase of entomophages applied in year				
		1991	1992	1993	1994	1995
Encarsia formosa	K	100	308	720	580	450
	G	100	260	1400	5900	13000
Phytoseiulus persimilis	K	100	290	450	200	110
	G	100	85	380	660	1000
Amblyseius cucumeris	K	100	100	300	40	40
	G	100	430	1000	4080	3430
Orius sp.	K	-	100	220	480	210
	G	-	-	100	170	310
Dacnusa sibirica *Diglyphus isaea*	K	-	-	100	140	100
	G	-	-	100	390	610
Aphidius colemani *Aphidoletes aphidomyza*	K	-	100	63	30	0
	G	-	100	540	1600	3200
Steinernema sp. *Heterorhabditis sp.*	K	-	-	-	-	100
	G	-	-	-	100	580

C - SLH farms F - other farms

Assessment of the potential market for beneficial species has been based on the rates of entomophages recommended by ROL-EKO /Table 3/. These rates correspond with average numbers of entomophages applied within SLH farms in 1994. Areas for the potential use of particular entomophagous species have been estimated upon the area of vegetable crops in 1994. In the case of nematodes parasites of insects, estimated areas of young plants and ornamental plants have been taken into account /Table 1/. However, the presented estimates could differ if the structure of crops changes.

Table 3. Potentiai market for entomophages in Poland.

Entomophagous species	Recommended rate per 1 m²	Treated area		Potential scale (in million)	Actual covering of market (in %)
		K	G		
E. formosa	12	220	1960	262	9,6
Ph. persimilis	12	220	240	55	6,4
A. cucumeris	400	20	230	1000	3,0
Orius sp.	1	20	230	2,5	1,0
D. sibiryca *D. isaea*	1,5	200	1200	21	1,2
A. colemani *A. aphidomyza*	3	25	840	26	1,2
Steinernema *Heterorhabditis*	500000	10	900	4500000	0,5

C - SLH farms F - other farms

The area under cover indicates that at least 2000 Ha of crops under the cover could be protected by the biological method. At present, entomophagous species are applied in ca. 90% of SLH farm areas, while in other farms no more than 10% of the area is subjected to this measure. Sale of entomophages expected in 1995, with regard to the potential demand for predators and parasitoids, forecasts the possible expansion of this market in Poland /Table 3/. Considering the prospects for the development of biological methods, the use of entomophagous nematodes in mushroom houses /200 Ha/ and integrated orchards /now above 2500 Ha/ should also be taken into account.

To complete the presented remarks, it should be remembered that the development of biological methods strongly depends on adequate qualifications of growers and extension people for modern horticultural production. On the other hand, such methods should be more economical as compared to chemical measures, since biological products are relatively more expensive than chemical pesticides. Also a low effectiveness of small greenhouse farms in Poland limits an interest in the biological method. However, the use of modern technologies /rockwool, bumble bees for pollination/ advances the introduction of these methods.

Role and Place of Microbiological protection in Agriculture and Forestry of Southern Russia

M.S.Sokolov, V.T.Goncharov

Department of Plant Protection of Russian Academy of Agricultural Sciences, All-Russian Research Institute of Biological Plant Protection, 350039 Krasnodar-39, Russia

Summary

In this paper the bases of three principal strategies of biological plant protection are stated. Role and place of microbiopreparations in the strategy of "operational hold-back" of injurious species are considered. An assortment of perspective microbiopreparations recommended to use in Russia is described. Experimental data and practical recommendations are produced on the use of these means for pest and disease control of such crops as winter wheat, corn, vegetables, tobacco, as well as tree plantations and shrubberies. Actual tasks in the field of applied science are formulated for research-workers developing, improving and creating microbiopreparations.

Introduction

The modern biological protection of agricultural plants as well as tree plantations and shrubberies is based on three traditional strategies (Соколов, Терехов, 1995). First, this is a long-term regulation of the number proportion between useful and harmful species, based on the rational use of agrarian and natural resources. As a result of its regulation, optimal correlation between useful, harmful and so named "indifferent" biota is formed within an agrolandscape. Secondly, this is a "self-protection" based on the innate resistance of varieties. Thirdly, this is an "operational hold-back" of pathogens, pests and weeds with the help of classical biomethod means and so named extensive biomeans or microbiopreparations. The first two strategies are more preferable because they are preventive by their nature. The ultimate decision on the application of the every concrete strategy or their combinations is taken after evaluating of economical threshold of harmfulness including state and number of natural of entomophages and other useful biota.

The introduction of biocontrol strategies starts with the thorough estimate of agrobiocenosis phytosanitary state. The final choice of harmful species, from which agricultural crops must be protected, is made by taking into account the results of phytosanitary monitoring carried on in consideration of biological, economical and social factors.

Assortment of Microbiopreparations

Microbiopreparations, together with classical biomethod means, are the most important element in the strategy of harmful species "operational hold-back" (Соколов, Терехов, 1995). The official list of microbiopreparations used in Russia for plant protection from pathogens and pests contains 83 preparations: 61 entomoacaricides and 22 preparations against mycoses, bacterioses and phytohelminthoses (Список ..., 1994; Биологические и биорациональные средства ... , 1995). They are produced centrally directed in integrated biological plants, in regional biofactories, and also directly in biolaboratories on farms. The active cultures of strain-producers are preserved in the All-Russian Collection of Microorganisms (Institute of Biochemistry and Physiology of Microorganisms, All-Russian Institute of Plant Protection (Каталог ..., 1992)) as well as within different research institutes by creators of these microbiopreparations. At present, dozens of preparations are being checked and tested under industrial conditions on farms before their official registration. The head institute for official registration of microbiopreparations (by toxicological-hygienical evaluation) is the State Scientific Research Institute of Applied Microbiology (Obolensk, Moscow Region).

According to their origin, the allowed to use microbiopreparations are divided into: **bacterial** - 37 (45%), (with BT-basis) - 28 (34%); **fungous** - 24 (29%); **viral** - 11 (13%); **actinomycetic** - 10 (12%); **nematodic** - 1 (1%).

Unlike protected ground conditions (vegetable greenhouses (Павлюшин, Соколов, 1994), the integrated biocontrol systems for open ground crops have not been developed up to now. The most effective methods of growing different agricultural crops without pesticide application were the subject under discussion at the All-Russian Conference (Экологически безопасные ..., 1994) and were generalised in "Regional Recommendations" (Производство экологически безопасной продукции ..., в печати).

The concrete examples of the successful use of microbiopreparations for pest and disease control of different agricultural crops, tree-plantations and shrubberies are given below.

Experimental Data and Practical Recommendations

1. Winter Wheat. Bacterial and actinomycetic biopreparations are effective against most pathogens infecting wheat seeds. In the absence of smut fungi they are widely used as means alternative to chemical treatment. For example, in comparison to TMTD and fundazole, rhizoplane provided a higher yield increase (2,7 metric centners/ ha and 3,3 metric centners/ha). Mycostop and bactophyte were even more effective (tables 1,2).

Table 1.

The effect of biopreparations at presowing winter wheat seed treatment (variety Olimpiya) on the reduction of harmfulness of *Fusarium graminearum* (natural infectious background, treatment at the flowering stage; All-Russian Scientific Research Institute of Biological Plant Protection, 1991-1992; Vyalyh A.K.)

Variant of the experiment (doze, kg/t)	Affected plants number, %	Ear affection degree, %	Grain yield, centner/ha, total	Grain yield, centner/ha, increase
1. Control	9.0	82	64.2	-
2. TMTD (4) (tetramethil-tiuran disulphide)	6.2	38	66.8	+2.6
3. Bactophyte (1)*	4.2	45	72.9	+8.6
4. Mycostop (1.5)**	5.1	48	68.4	+4.2
5. Rhizoplan (2)***	6.3	64	66.9	+2.7

Titres of culture liquid: *)$2.2 \cdot 10^9$; **)$2.4 \cdot 10^9$; ***)$2.9 \cdot 10^9$

Table 2.

The efficiency of Rhizoplan at winter seed treatment of variety Scythianka (Joint-stock company "Privolnoye" of Krasnodar region, 1994-1995; V.T.Goncharov)

Variant of experiment	Preparation discharge rate per 1t of seeds	Affection degree of plants by leaf spots, %	Yield of raw grain, centner/ha	Increase in yield, centner/ha
Control	-	30	25.7	-
Rhizoplan	1 l	13	30.3	4.6
Fundazol (standart)	3 kg	14	29.0	3.3

In the South of Russia by epiphytoties of head blight (*F.graminearum*) microbiopreparations provide moderate protection from this pathogen. Although by artificial infection they were less effective than folicure - the best systemic fungicide, however they acted just as good as contact pesticides (policarbacin) (table 3).

By the treatment of vegetative plants on the natural infection background, the antifusarious activity of bacterial, actinomycetic and fungous biopreparations (by evaluation of plant productivity and thousand-seed-weight) was not inferior to the activity of standard fungicide tilt (table 4). Moreover, such biopreparations as alirin C and trichotecin provided the vomitoxin content decrease in grain by 25%.

Table 3.

The efficiency of microbiopreparations against *F.graminearum* (artificial infectious background, small plot experiments in winter wheat of variety Spartanka in the greenhouse and in the field, inoculation - at the end of flowering; 1994-1995, V.M.Androsova)

Variant of the experiment (dose, kg/ha)	Biological activity, %
1. Folicur (1.0)	84
2. Alirin C (3.0)	52
3. Immunophyte (ethylic ether of arachidonic acid) (0.3 g/ha)	49
4. Mycostop (2.0)	49

Table 4.

Grain yield formation of winter wheat (Spartanka variety) at the background of natural infection with *F.graminearum* (small plot experiment in the state farm "Vtoraya Pyatiletka" of Leningrad region, treatment at flowering stage, 1992-1993; A.K.Vyalyh)

Variant of the experiment (culture liquid dilution)	Ear mass, g	Mass of 1000 grains, g	Germinating power, %
1. Control	1.26	36.5	77
2. Tilt (0.5 kg/ha)	1.40	39.4	98
3. Bactophyte (Bac. subtilis*)			
(1:1)	1.58	41.0	88
(1:10)	1.36	39.0	89
4. Trichotecin (Trichotecium roseum)			
(1:1)	1.81	39.0	88
(1:10)	1.45	39.5	82
5. Alirin B (Bac. subtilis**)			
(1:1)	1.42	41.0	92
(1:10)	1.45	42.0	95
6. Alirin C (Streptomyces felleus***)			
(1:1)	1.51	40.1	92
(1:10)	1.44	42.0	95

Titres of culture liquid: *)$2.2 \cdot 10^9$; **)$2.4 \cdot 10^9$; ***)$2.6 \cdot 10^9$

2. Grain corn. By protection of this culture from *Ostrinia nubilalis Hbn*, the preparations with BT-basis received the greatest application. According to the data obtained by V.F.Kobsar and co-authors (Кобзарь с соавт., 1994), the two-fold aerial application of lepidocide-100 with the spray liquid consumption 50 l/ha provided a high protective effect (82%) and the greatest grain yield increase - 16,8 metric centners/ha (table 5).

Table 5.

The efficiency of lepidocid-100 application at cornprotection from stem moth (*Ostrinia nubilalis Hbn*); V.F.Kobzar and co-authors, 1994.

Variant of the experiment at the discharge per 1 ha: suspension, l/ preparation, kg	Replications number	Individuals dead rate, %	Protective effect, %	Grain yield, centner/ha	Increase in yield, centner/ha
Control	-	12	-	68.5	-
25/1.5	1	46	-*)	-	-
25/1.5	2	60	-	-	-
50/1.5	1	50	77	72.7	+4.2
50/1.5	2	67	82	85.3	+16.8
25/3.0	1	45	-	-	-
50/3.0	1	50	-	-	-
			LED_{05}	6.8	

*) - the protective effect was not determined
LED - The lowest essential difference

3. Vegetable crops. According to the recommendations of scientific workers of the Krasnodar Research Institute, biopreparations can be used both for the presowing treatment of vegetable crop seeds against phytopathogenes and for the treatment of vegetative plants against pests and pathogens (Голубев и соавт., в печати). Cabbage seeds should be treated against fungous diseases with trichodermin (0,3-0,4 kg/t seeds, 24 hours before sowing). With the same purpose, tomato seeds are treated before sowing with rhizoplane (in 0,01% of the preparation water suspension they are recommended the application of boverin (4 l/ha), metarizin (10 l/ha) and feeding concentrate of lisin with granulated superphosphate against onion fly and click beetle larvae. Against downy mildew and neck rot of onion rhizoplane recommended; the first treatment should be made during the 4-6-leaf stage, the two next ones - at intervals of 10 days.

Against the larvules of blue fleas and cabbage moths lepidocide (1 kg/ha) or bitoxibacillin are recommended to use. To control Alternaria leaf spot, black bacterial spot and foot rots of cabbage the two or three-fold treatment with rhizoplane (1 kg/ha) or fungistop (2-3 l/ha) should be made.

Among microbiopreparations against meadow moth caterpillars, brassy flea beetles, aphides and weevils bitoxibacillin (2 kg/ha) and lepidocid-100 (0,6-1 kg/ha) are recommended for application. The first treatment is made during the seedling stage by a number of fleas 0,2 individuals/plant and more. The treatment should be repeated in 7-10 days. The third treatment of red beet fields is to be made within the rosette stage.

On tomatoes bitoxibacillin, or BTB (2 kg/ha) is recommended to apply to control Colorado potato beetle. The preparation is effective against younger ages of this beetle 5-8% of colonised plants and density 2-3 larvae/plant. Biological effectivity of BTB for larvae - 85%, for egg layings - 87%. The first treatment against Colorado potato beetle should be made at the period of egg laying - beginning of the first generation larvae appearance, 7-8 days later follows the second treatment. The third BTB-treatment is made during the egg-laying - second generation larvae appearance period. If it will be necessary, the forth treatment can be done (not later than 2 days before harvesting). For the bollworm control lepidocid is recommended to use (1 kg/ha), its biological effectivity for cotton-boll worms of the first and second age is 75%. The first treatment against boll-worms should be made at the period of the first generation larvae appearance, then it should be repeated in 6-7 days. The third treatment follows after the second generation larvae appearance; if necessary the fourth treatment also can be made.

By the protection of tomatoes from the complex of pests it is advisable to alternate BTB with lepidocide. From the complex of diseases tomatoes are protected by treatment with 0,005% of rhizoplane suspension (1,2-5 l/ha) at the time period, when plants reach 15-20 cm. The second and all the next treatments should

be started from the flower bud stage or by the first signs of late blight (at intervals of 10-14 days). It is effective to alternate rhizoplane sprays with the 1% Bordeux liquid treatment.

4. Tobacco. Microbiopreparations (trichodermin) have shown a high effectivity (by separate or combined action) by the protection of tobacco seedlings from the complex of root rots caused by fungous phytopathogens (*Pythium spp., Rhizoctonia spp., Fusarium spp.* - table 6). It is significant, that some substrates improving suppressive properties of outdoor seed bed soils (such as alfalfa flour, fallen oak leaves, winter rape as siderat) provided practically complete protection from root rots, while the fungicides (avixil, rhidomil, fundazole) were less effective (table 7). Possibly, it can be explained with the induced resistance of the above-mentioned pathogens to conventional fungicides.

Table 6.

The efficiently of tobacco seedling protection methods from causative agents of rot (*Pythium spp., Rhizoctonia spp.*) (All-Russian Scientific Research Institute of Tobacco and Tobacco Goods, 1995; O.D.Phylipchuk)

Variant of the experiment	Seedling affection, %		Biological efficiency, %	
	natural background	Rhizoplan 15 ml/m^2	natural background	Rhizoplan 15 ml/m^2
Control	30	-	-	-
Avixil, 70% wettable powder, 0,3 g/m^2	6	-	80	-
Trichodermin, 15 ml/m^2 (autumn application)	0	0	100	100
Alfalfa, 350 g/m^2	0.5	0.8	98	97
Oak-tree litter-fall, 150 g/ m^2	0	0	100	100
Winter rape, 1300 plants/ m^2	0.6	0.6	98	98
Trichodermin, 15 ml/m^2 (two weeks before sowing)	8	2	74	94
Trichodermin, 15 ml/m^2 (on a dav of sowing)	23	7.5	24	75
LED_{05}	7.0	1.4		

LED - The lowest essential difference

Table 7.

The efficiency of biopreparations against the causative agents of tobacco seedling rot (All-Russian Scientific Research Institute of Tobacco and Tobacco Goods, 1994; O.D.Phylipchuk)

Variant of the experiment	Seedling affection, %		Biological efficiency, %	
	Pythium spp.	Fusarium spp.	Pythium spp.	Fusarium spp.
Control	36	33	-	-
Ridomil, 1g/m^2 + Fundasol 3 g/m^2	11	16	71	51
Bactophyte, 15 ml/m^2	10	12	72	62
Trichodermin, 15 ml/m^2	9	9	76	72
Rhizoplan, 15 ml/m^2	10	12	72	62
LED_{05}	3.2	3.1		

LED - The lowest essential difference

All the biopreparations without exception were not inferior in their effectivity to such a widely recommended incectonematicide as counter (table 8).

Table 8.

The efficiency of biopreparations against click beetles (*Agriotes spp.*) on tobacco plantations (The experimental field of Abinsk, 1993; O.D.Philipchuk)

Variant of the experiment, kg (l)/ha	Tobacco plants number, pieces			Biological efficiency, %	
	planted	injured (at calculations)		1	2
		1	2		
Control	181	22	25	-	-
Caunter, 5%, 30	181	8	9	64	64
Lepidocid, 4	179	8	8	64	64
Bitoxibacillin, 4	179	8	9	64	64
Metarizin *), 10	179	7	7	68	72
Boverin, 4	181	7	7	68	72
LED_{05}	7	1	1		

LED - The lowest essential difference

*) Producer - *Metarrhizium anisopliae*

5. Tree plantations and shrubberies. The preparations with the BT-basis (lepidocid, bitoxibacillin, dendrabacillin, gomelin) are widely used for the protection of valuable mountain forests, for example, oak forests, from the complex of pests. Among them are fall webworm moth (*Hypantria Cunea Drury*), gispy moth (*Ocneria dispar L*), *Tortix virudana* and complex of *Geometridae spp.* By the small volume aerial application, the effectivity of microbiopreparations comes to 80-100% (tables 9, 10).

Table 9.

The efficiency of biopreparations aerial application against the American white moth (V.F.Kobzar and others, 1991)

Preparation, form, titre, billions/g	Discharge, kg/ha	Indecies of Biological efficiency	
		dead rate with the correction for control according to Abbot, %	tree crown state, K_i
	1986 (Krasnodarsky	mechanized forestry farm)	
Stabilized Lepidocid, 100	0.8	93.0	0.89
Bitoxibacillin, wettable powder, 60	1.5	100	0.89
Bitoxibacillin, wettable powder, 45 (standart)	2.0	100	0.86
	1987 (Krasnodarsky	mechanized forestry farm)	
Stabilized Lepidocid, 100	0.8	83.6	0.92
Bitoxibacillin, wettable powder, 60	1.5	84.0	0.90
Bitoxibacillin, wettable powder, 45 (standart)	2.0	the calculation has not been realized	0.66
	1988 (Terskiy	forestry farm)	
Stabilized Lepidocid, 100	0.8	80.0	0.95
Bitoxibacillin, wettable powder, 60	1.5	98.3	0.97
Bitoxibacillin, dry powder, 45 (standart)	2.0	91.0	0.90

Table 10.

The efficiency of the reduced norms of microbiopreparation suspension discharge (V.F.Kobzar, 1991)

Preparation (titre, form)	Discharge rate per 1 ha		Caterpillars dead rate, %		
	suspension, l	preparation, kg	Gyspy moth (Ocneria dispar)	Complex of inch-worms	Oak green leaf roller moths
Dendrobacillin, wettable powder[1)]	25	1.5	-	94.7	-
Dendrobacillin, wettable powder[1)] + Dimilin, 25% wettable powder	25	1.0	-	91.7	-
Dendrobacillin, wettable powder[2)]	25	1.0	100	-	-
Dendrobacillin, wettable powder[2)] + AE-4P (AE=anti-evaporizer)	25	1.0	91.7	-	-
Dendrobacillin, wettable powder[3)]	25	1.0	-	-	94.7
Dendrobacillin, wettable powder[4)]	50	3.0	100	92.9	-
Gomelin, wettable powder[3)]	25	1.0	90.9	-	94.1
Gomelin, wettable powder[3)]+ urea	25	1.0	93.8	-	-
Gomelin, powder[4)]	50	2.0	91.7	-	-
Lepidocid[3)]	25	0.8	100	-	89.2

Titres of preparations: [1)] - $60 \cdot 10^9$; [2)] - $82 \cdot 10^9$; [3)] - $100 \cdot 10^9$; [4)] - $30 \cdot 10^9$

Conclusion

The modern biocontrol disposes of a great arsenal of biological means including useful organisms, their products and metabolites, pathogenic viruses of animals and bacteriophages. In Russia nearly 20% of all protected areas are treated by these means, with the share of microbiopreparations - about 10%. To the reasons limiting biocontrol means application belong the lack of investments (from the State and sponsors) as well as the absence in our country of real economical stimulation for ecologically safe plant production.

Up to the present, no biocontrol system has been created, that would allow to obtain acceptable yields of economically significant crops without the use of wide-spectrum agrochemicals. Research in the field of the leading biocontrol strategy - the long-term agrobiocenotic regulation of agrolandscape biota on a limited scale. At least, practically no recommendations on the use of mycoherbicides to control weeds and narcotic plants are available.

To solve the above-mentioned problems coordinated intensive efforts of scientists in different countries are necessary, including their active participation in the development of large-scale international projects. The specialists of our Institute are ready to take part in all the efforts of this kind.

References

Биологические и биорациональные средства защиты растений. Краткий справочник, 1995 /Э.А.Кобилева, С.А.Ермоленко, Н.Ф.Шевченко, Т.П.Винокурова, А.П.Еременко. Под ред. д.с.-х.н. В.Ф.Кобзаря. Краснодар, ВНИИБЗР, 42 с.

Голубев А.Я., Артюшенко Н.А., Болахоненков В.Е., Варварина Н.А., Титаренко Л.Н., Иванова В.Д., Алешенко М.Н. (в печати). Технология возделывания овощных культур на Кубани. Производство экологически безопасной продукции растениеводства. Пущино, ВНИИБЗР.

Каталог культур микроорганизмов, 1992. Пущино-Москва, ИБФМ РАН, 362с.

Кобзарь В.Ф., 1991. Теоретические основы и разработка авиационного способа применения бактериальных препаратов против хвое- и листогрызущих насекомых. Автореф. диссерт. д.с.-х.н. Л.: 26

Кобзарь В.Ф., Ширяева Н.В., Чирков В.М. 1991ю Применение лепидоцида и битоксибациллина против американской белой бабочки. Лесное хозяйство, N1: 54-55.

Кобзарь В.Ф., Пушин В.Г., Устименко А.А., 1994. Защита биосредствами посевов кукурузы от стеблевого мотылька в условиях орошаемого земледелия. Экологически безопасные и беспестицидные технологии получения растениеводческой продукции. Пущино, ВНИИБЗР: 7-12.

Павлюшин В.А., Соколов М.С., 1994. Предварительные итоги работы по проекту 03 (овощеводство защищенного грунта). Экологически безопасные и беспестицидные технологии получения растениеводческой продукции. Пущино, ВНИИБЗР: 41-45.

Производство экологически безопасной продукции растениеводства. Региональные рекомендации (в печати). Под ред. М.С.Соколова, Е.П.Угрюмова. Пущино, ВНИИБЗР.

Соколов М.С., Терехов В.И., 1995. Современная концепция биологической защиты растений. Агрохимия, N4: 90-98.

Список химических и биологических средств борьбы с вредителями, болезнями и сорняками, регуляторов роста растений и феромонов, разрешенных для применения в сельском, в том числе фермерском, лесном и коммунальном хозяйствах, на 1992-1996 гг., 1994, М.: Колос. 318 с.

Филипчук О.Д., Ярошенко В.А., Исмаилов В.Я., Вяткина Г.Г., 1995. Эффективность биологических и химических препаратов против вредителей табака. Агрохимия, N8: 92-97.

Экологически безопасные и беспестицидные технологии получения растениеводческой продукции. 1994. Материалы Всероссийского научно-производственного совещания (Краснодар, 24-26 августа 1994 г.) ч.1 178 с., ч.2 271 с.

2. Microbial Control of Forest Pests

THE CONTROL OF THE NUN MOTH *LYMANTRIA MONACHA* L. WITH THE USE OF *BACILLUS THURINGIENSIS* BERL. IN POLAND

B.GLOWACKA

Department of Forest Protection, Forest Research Institute,
Bitwy Warszawskiej 1920 r. 3, 00-973 Warsaw, Poland

1. SUMMARY

Scots pine defoliating insects are major economic pests of forest stands in Poland. Larvae of *Lymantria monacha*, *Panolis flammea*, *Bupalus piniarius*, *Dendrolimus pini* and *Diprionidae* require control treatments on many thousand hectares almost every year.

The nun moth *Lymantria monacha* appears in outbreaks at Scots pine, larch and spruce stands. In the past it was controlled many times mainly with the use of organochlorines and pyrethroids. At the same time, investigations were carried out on the control of the nun moth with the use of insecticides more friendly to the environment including the *B.thuringiensis* products.

In control treatments, however, *B.thuringiensis* was used on a small scale. During the biggest outbreak in 1978-1984 when the nun moth was controlled on a total area of 6 million ha, *B.thuringiensis* was tested on 50 000 ha of treated stands only, while 99,2% of infested stands were sprayed with pyrethroids.

Since 1991, the increase in the density of the nun moth population has been observed. In 1994 the project "The integrated control of nun moth in Polish forests" was developed and financed by own and foreign funds. The use of pyrethroids was strongly reduced, they were applied at 74 600 ha of forest stands only. *B. thuringiensis* was used at 150 000 ha and diflubenzuron - at 522 300 ha.

The % mortality of the nun moth calculated after 4 weeks following the treatments approximated 100%. There was no statistically significant difference in the insecticidal activity of *B.thuringiensis*, diflubenzuron and pyrethroids.

2. FIELD EXPERIMENTS WITH *B. THURINGIENSIS* PRODUCTS

The studies on the use of the *B.thuringiensis* in the nun moth control treatments were started in Poland in the '70s. They followed by the 20-year control field tests with the use of about twenty commercial products specified in table 1.

Table 1. *B.thuringiensis* products tested against the nun moth

Insecticide	Producer
Bacilan	ZPR Walcz, Poland
Bactospeine 16000	Biochem, Belgium
Bactospeine creme 6000	Biochem, Belgium
Bactospeine creme 8000	Biochem, Belgium
Bathurin	JZD Agrokombinat, Czechoslovakia
Biospor	Hoechst AG, Germany
Bitoksybacillin	Glavmikrobioprom, Soviet Union
Dipel WP	Abbot Lab. USA
Dipel 8L	Abbot Lab. USA
Dendrobacillin	Glavmikrobioprom, Soviet Union
Ecotech Pro 7,5	Ecogen, USA
Ecotech XL	Ecogen, USA
Entobakterin	Glavmikrobioprom, Soviet Union
Foray 48B	Novo Nordisk, Denmark
Foray 76B	Novo Nordisk, Denmark
Gomelin	Glavmikrobioprom, Soviet Union
Lepidocyd	Glavmikrobioprom, Soviet Union
Thuridan WP	Polfa-Pabianice, Poland
Thuridan krem	Polfa-Pabianice, Poland
Thuricide 90TS	Stauffer Chem. Comp., USA
Thuricide WP	Bioferm Corp., USA

Initially the tested insecticides were applied in the form of water dispersable powders with the use of ground or aerial hydraulic sprayers, at a dose of 1-2 kg mixed with 50-100 l water per 1 ha. Their use caused much trouble and the efficacy was often insufficient. Later on the ULV formulations of *B. thuringiensis* with the use of atomisers were applied at a dose 1-4 l of the product per ha.

3. USE OF *B.THURINGIENSIS* FOR NUN MOTH CONTROL

B. thuringiensis products were first used on a larger scale in the protection of forests against the nun moth in 1982-1984. Control treatments were then carried out over a total area of about 50 000 ha in national parks, recreation forests, and in the neighbourhood of water reservoirs.

Two formulations Bactospeine creme and Thuridan creme (table 2), were applied at a dose of 1-2 l mixed with 5-50 l of water per 1 ha.

The obtained efficacy varied and to the great extent depended on species composition of the treated stands. At Scots pine stands it was generally higher (80-100% mortality of caterpilars) while at spruce stands the control efficacy was much lower (30-80%).

Table 2. *B.thuringiensis* products used in the nun moth control treatment

Insecticide	Treated area	Mortality (%)
Bactospeine creme 6000	26 000 ha	30-80
Bactospeine creme 8500	20 000 ha	60-90
Thuridan krem	5 500 ha	60-90
Foray 48B	150 000 ha	75-100

B. thuringiensis was for the second time used in a nun moth control treatment in 1994.

The project "The integrated control of nun moth in Polish forests" was developed and financed by own and foreign funds. The infested forest stands were treated with aircraft equipped with a new type of atomiser Micronair AU 5000. Spraying of Scots pine stands was started as soon as the nun moth larvae hatched. The share of pyrethroids (Decis 2,5 EC, Fastac 10 EC, Trebon 10 SC) was strongly reduced, they were applied on 74 600 ha of forest stands. *B. thuringiensis* (Foray 48 B) was used on 150 000 ha (table 2) and diflubenzuron (Dimilin 480 SC) - on 522 300 ha.

The % mortality of nun moth larvae approximated 100%. There was no statistically significant difference in the insecticidal activity of Foray 48 B, Dimilin 480 SC and pyrethroids.

4. EFFECTS OF *B.THURINGIENSIS* PRODUCTS ON NON-TARGET ARTHROPODS

In field trials in 1993 and in control treatments in 1994, the direct effect of pyrethroids, diflubenzuron and *B. thuringiensis* products on non-target arthropods (insects and spiders) was compared. Parasitoids, predators and other arthropods were collected on $1m^2$ linen cloths placed beneath the trees treated with insecticides. Table 3 shows the mean numbers of beneficial and economically indifferent arthropods found on the linen cloths.

Statistical analysis indicated that there are no significant differences in the mortality of these arthropods on

the trees treated with *B.thuringiensis*, diflubenzuron and on untreated trees.

Table 3. The mean number of beneficial and economically indifferent arthropods per 1 m^2

Year	Insecticide	Specimens		
		Parasitoids and predators	Indifferent	Total
1993	Fastac 10 EC	18,25	5,35	23,60
	Sumi-Alpha 0,5	8,74	5,60	14,34
	Foray 48B	0,64	0,65	1,29
	Dipel 8L	0,45	0,90	1,35
	Dimilin 45 ULV	0,76	2,16	2,92
	Untreated	0,80	1,80	2,60
1994	Decis 2,5 EC	13,00	24,60	37,60
	Trebon 10 SC	15,60	21,60	37,20
	Foray 48B	1,00	2,20	3,20
	Dimilin 480 SC	1,20	2,40	3,60
	Untreated	0,60	3,80	4,40

The data proves a strong negative side effect of pyrethroids on non-target arthropods. In the stands treated with pyrethroids the mortality of non-targets calculated from dead-dropping beneficial insects, spiders and economically indifferent insects was approximately 10 times higher than mortality in the stands treated with *B.thuringiensis* and diflubenzuron.

Degradation of *Bacillus thuringiensis* serovar *kurstaki* after aerial application to a Polish pine stand

Per H. Damgaard[1], Henryk Malinowski[2], Barbara Glowacka[2] and Jørgen Eilenberg[1]

[1]Department of Ecology and Molecular Biology, Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C., Copenhagen, Denmark, [2]Forest Research Institute, Department of Forest Protection, ul. Bitwy Warszawskiej 1920 r., No 3, Warsaw, Poland

SUMMARY:

During the spring of 1994 an extensive spraying campaing using *Bacillus thuringiensis* against *Lymantria monacha* (the Nun Moth) in Polish forests was implemented. Before spraying, a total of 35 *B. thuringiensis* strains were isolated from needles of Scotch Pine. The strains were found to belong to seven different serotypes, including *kurstaki* and *israelensis*. On day 1, 7, 28, 56 and 121 after spores and crystals of *B. thuringiensis* serovar *kurstaki* (Foray 48B) were applied, the spores were isolated from collected needles by plate spreeding. The initial degradation rate ($t_{½}$) of the applied BtK spores on Scotch Pine needles was found to be 1.75 days, and spores could still be detected in concentrations of 4×10^2 spores/g 121 days after application. These concentrations are significantly higher than the natural background population of *B. thuringiensis* found on the needles prior to spraying. Bioassays using *L. monacha* (L_2) have shown that no measurable insecticidal activity was left on the needles 21 days after spraying, although spores could still be detected by plate spreading.

INTRODUCTION:

Outbreaks of *Lymantria monacha*, a polyphagous forest defoliator, occur regually in Scotch Pine and spruce in Poland. The last outbreak was in 1978-1984 and was mainly controlled with chemical pesticides. In 1991 a new outbreak started and in 1994 it was decided to use *Bacillus thuringiensis* on 20% of the 743 000 ha of forest sprayed.

B. thuringiensis is the most used microorganism for insect pest control and is generally considered to be an environmentally safe microbial pesticide, but the environmental side effects of spraying, including disturbance of the native *B. thuringiensis* population, are still poorly understood. The aim of this study was to get more information about the possible interactions between a sprayed *B. thuringiensis* based pestcide and the native *B. thuringiensis* population present on pine needles.

MATERIALS & METHODS

Location

In the forest district Ostrów Mazowiecka *B. thuringiensis* serovar *kurstaki* (Foray 48B) was applied to a Scotch Pine stand by aerial spraying (4 ℓ/ha.)

Sampling

Samples of pine braches were collected from the experimental forest plot 3 days before spraying and on day 1, 7, 28, 56 and 121 after application.

B. thuringiensis isolation

3 g pine needles were placed in sterile Stomacher bags with 10 ml PBS and blended for 3 x 30 sec. at high speed in a Stomacher 80 Lab-blender. The supernatant was collected and centrifuged (25 min., 4300 x g), and the resulting pellet was resuspended in 1-5 ml PBS and heat-treated for 30 min at 65 °C in a waterbath. Ten-fold serial dilutions of the heat treated suspensions were plated on T_3 -sporulation agar (Travers *et al.* 1987) and incubated at 30°C for 24 h. Colonies with a rugose and "ice-crystal" appearance and a diameter > 2 mm were selected and subcultured on T_3 until sporulation. Isolates were examined using a phase-contrast microscope, and cells containing a parasporal inclusion body (crystal) in the sporangium were classified as *B. thuringiensis*, whereas the rest were classified as *B. cereus*.

Serotyping

Serotyping was performed as described by de Barjac (1981) , using the antisera against the 58 currently recognised serotypes (serovars) (M. Lecadet, personal communication, May 1994).

Bioassays

30 *Lymantria monacha* (L_2) larvae in tree replicates were placed on branches of pine collected from the sprayed plot on day 1, 5, 7 and 14 after application. Mortality of the larvae was recorded after 8 days.

RESULTS & DISCUSSION

Out of 43 samples of needles collected prior to spraying, 20 samples were found to contain at least

one *B. thuringiensis* isolate. The frequency of *B. thuringiensis* was found to be 0.07 among the total *B. thuringiensis*/B. *cereus* population. A total of 35 *B. thuringiensis* strains were isolated giving an abundance of ≤ 0.5 - 5 *B. thuringiensis* spores / g needles. From the naturally occurring strains, 3 different morphotypes of crystals inclusions were found: bipyramidal, square or rund/irregular. Tabel 1 shows that 24 out of the 35 isolated strains had round or irregular crystals and among those, most strains belonged to serotype *israelensis*. Strains belonging to serotype *israelensis* have later been shown by SDS-PAGE electrophoresis to contain proteins (protoxins) of the size expected to be active against dipterans. The serotype *kurstaki* was found in all three crystal morphology groups. It is noticable that strains of serotype *kurstaki* was found to be naturally occurring on the needles prior to application of Foray 48B which consists of serotype *kurstaki* spores. As only 35 strains have been isolated so fare, it is not possible to draw conclusions about the relative abundance of the different serotypes or morphology groups.

Table 1. Crystal morphology and serotype of *B. thuringiensis* isolated from needles prior to spraying.

Crystal morpho-logy	Bipyramidal			Square		Round/irregular						
Serotype	KUR	AIZ	NIG	KUR	UTY	KUR	OST	ISR	AMA	AND	TOC	UTY
Number of strains	1	7	1	1	1	1	1	11	1	1	1	8

AIZ: *aizawai*; AMA: *amagiensis*; AND: *andalousiensis*; ISR: *israelensis*; KUR: *kurstaki*; NIG: *nigeriensis*; OST: *ostriniae*; TOC: *tochigiensis*; UTY: untypable by reference H antisera against serotypes 1-45.

The number of BtK spores occurring on the needles after application was found to decrease with time (Table 2). The initinal half-life of the spores ($t_{½}$) was calculated to be 1.75 days, which is in accordance with other reports on similar *B. thuringiensis* products with slight variations between the different plant species treated (Pinnock *et al.* 1971; Pinnock *et al.* 1974; Pinnock *et al.* 1975). The data also show ca. 4 x 10^2 spores / g could still be isolated from the needles 121 days after aerial application, concentrations significantly higher than the natural *B. thuringiensis* background population.

Table 2. Number of *B. thuringiensis* spores detected on pine needles after application of Foray 48B.

Days after application	*B. thuringiensis* spores / g needles (mean)
1	1.4×10^5
7	1.5×10^4
28	4.2×10^3
56	4.4×10^2
121	4.3×10^2

Table 3. Mortality of *L. monacha* (L_2) placed on pine branches collected after aerial application of Foray 48B.

Days after application	Corrected mortality (%)
1	90
5	80
7	67
14	20

Table 3 shows that good control of the *L. monacha* larvae can be achived within the first week of application, giving a spraying window of approximately two weeks. After two weeks, the insecticidal activity of the product had almost stopped under the field conditions given in this experiment. This is in accordance with results from other studies on other similar products ("Thuricide" (Ignoffo *et al.* 1974), "Dipel" (Morris, 1977; Beegle *et al.* 1981)). In those cases, the insectcidal activity under field conditions had stopped within two weeks of application depending, on type of foliage treated, concentration used and target pest species and instar.

Future studies will focus on the degradation of the applied *B. thuringiensis* serotype *kurstaki* spores following day 121 after application, to document if the level of BtK residues will drop to the initial level of natural *B. thuringiensis* occurrence.

REFERENCES:

Barjac, H.d. (1981). Identification of H-serotypes of *Bacillus thuringiensis*. In *Microbial control of pests and plant diseases (1970-1980)* (Burges, H.D., ed.), Academic Press, London, pp. 35-43.

Beegle, C.C., Dulmage, H.T., Wolfenbarger, D.A. & Martinez, E. (1981). Persistence of *Bacillus thuringiensis* Berliner insecticidal activity on cotton foliage. *Environmental Entomology* **10**, 400-401.

Ignoffo, C.M., Hostetter, D.L. & Pinnell, R.E. (1974). Stability of *Bacillus thuringiensis* and *Baculovirus heliothis* on soybean foliage. *Environmental Entomology* **3**, 117-119.

Morris, O.N. (1977). Long term study of the effectiveness of aerial application of *Bacillus thuringiensis*-acephate combination against the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *The Canadian Entomologist* **109**, 1239-1248.

Pinnock, D.E., Brand, R.J. & Milstead, J.E. (1971). The field persistence of *Bacillus thuringiensis* spores. *Journal*

of Invertebrate Pathology **18**, 405-411.

Pinnock, D.E., Brand, R.J., Jackson, K.L. & Milstead, J.E. (1974). The field persistence of *Bacillus thuringiensis* spores on *Ceris occidentalis* leaves. *Journal of Invertebrate Pathology* **23**, 341-346.

Pinnock, D.E., Brand, R.J., Milstead, J.E. & Jackson, K.L. (1975). Effect of tree species on the coverage and field persistence of *Bacillus thuringiensis* spores. *Journal of Invertebrate Pathology* **25**, 209-214.

Travers, R.S., Martin, A.W.P. & Reichelderfer, C.F. (1987). Selective process for efficient isolation of soil *Bacillus* spp. *Applied and Environmental Microbiology* **53**, 1263-1266.

THE WAYS TO DECLINE THE EXPENDITURES OF BIOLOGICAL PREPARATIONS FOR FOREST PESTS CONTROL WITH THE METHOD OF EARLY DIAGNOSIS OF THEIR NATURAL DISEASES

N.V. Lappa

Institute of Plant Protection (Ukr.Akad.Agr.Sci), Ukraine, Kiev

Abstract

The methods of early diagnosis of diseases of forest pests, including haematological method were developed and certified. The analysis of the physiological state and occurrence of native entomopathogens and the diagnosis of early stages of diseases in population during allow us to choose microbial agents most suitable for the situation, that will not cause interference with native and released pathogens.

The relationships between the compounds of the forest biocoenosis are complex. To preserve useful species every effect to noxious compounds of the biocoenosis should be preceded by monitoring. The analysis of the physiological state and the occurrence of native entomopathogens allow us to choose microbial agents most suitable for the situation, which will not cause any interference of native and released pathogens.

It is shown that in the case of phytophagous populations weakened by natural diseases, especially protozoan, a significant deduction in the rate of treatment with biological and chemical preparations is possible (Lappa, 1961; Weiser, 1972; Issi, 1972), What is more, the higher the proportion of entomopathogens, capable of causing epizootics, in populations, the more possible it is to limit the control, localize it or reject it all together.

To localize disease agents methods of early diagnostics such as analysis of haemolymph and gut pH, and imprint preparations of various organs and tissues, as well as the method of serological diagnostic were developed and certified.

This paper presents the principles of the haematological method of evaluating the viability of phytophagous insects on the basis of the histological and microbiological pictures of their haemolymph. The fixed preparations of haemolymph painted by the method of Romanovksy-Gimsa were examined.To evaluate the weakness of insects the use of total amount of pathological and dead cells, as well as the nature and depth of the pathological changes, are preferable in comparison with the percentage of different groups of haemocytes. It is shown that some pathological changes in the haemolymph characteristic for the species, other features of the pathology are characteristic for separate diseases.

We define four types of pathological changes in haemolymph, in increasing order of of pathological signs. The first type: normal cells are prevailing, pathological changes are single, the proportion of dead cells does not exceed 3-5%. The second type: the proportion of pathological cells does not exceed 10-15%, dead haemocytes are relatively few. The third type: pathological changes are pronounced, the proportion of degenerative haemocytes is high (about 90%). The fourth type: there are deep pathological changes in most cells, as well as disease agents. Such gradation is acceptable for the examination of many phyllophagous lepidopterans.

So, in examining some sample groups of *Malacasoma neustria* L. caterpillars from various locations, we selected four micropopulations of the pest on basis of the picture of their haemolymph, They appeared to have all types of pathology as described above.

In the caterpillars of the first, most viable micropopulation, 86% of individuals had no signs of degenerative cells or had single ones. There were no caterpillars with the deep pathology of haemolymph. Also, there were no disease agents in the haemolymph. The following rearing of the pest showed slight death of caterpillars and pupae (5% and only 3% of them due to diseases). The butterflies had high fecundity.

In the second micropopulation of *M. neustria*, caterpillars of second and third types of pathology (52 and 42% respectively) were prevailing. 14% of individuals had the disease agents: microsporidia of the genus *Plistophora* and nuclear polyhedrosis virus. During the rearing 56% of caterpillars and pupae died, including 54% due to diseases. The rest of individuals became adult.

The third micropopulation was intermediate between the first and second one; most of the caterpillars showed first and second types of pathology (36 and 38% respectively); 10% of individuals had deep pathological changes of haemocytes besides the nuclear polyhedrosis virus. The following rearing showed 26% of death at the stages of caterpillar and pupae.

In the fourth micropopulation of caterpillars there were no insects with the first type of pathology. Most of the individuals had third and fourth types of pathology, i.e. the types with the deepest pathological changes and with the disease agents (bacteria, yeast, viruses) in haemolymph. The following rearing showed the death of most of the caterpillars (96%) due to diseases; emergent adults had low fecundity.

As one can see, there is a definite connection between the haematological parameters and the survival of the insects in the subsequent rearing. So, it allows for the use of the data of haemolymph analysis for diagnosis.

To provide the preliminary prediction the most acceptable stage for microbiological examination was chosen. It was shown that to discover preliminary disease and to plan control measures it is better to examine overwintering larvae in *Euproctis chrysorrhoea* L. and *Aporia crrataegi* L.; pupal stage in winter in *Hyphantria cunea Drury*; small larvae in *Malacosoma neustria* L.; ovipositions, previously incubated at optimal temperatures in *Ocneria dispar* L.

From 1957 we have been examining various populations of lepidopterous pests from forest locations in the Ukraine in order to discover the natural diseases and to define the expediency of planned protective measures. To find the early stages of diseases histological and microbiological indexes of haemolymph imprint preparations of various organs and tissues of insects were used. On the base of thorough microbiological and physiological examinations and taking into account the ecological factors it was recommended not to carry out the planned insecticide treatments in some locations. So, for *Euproctis chrysorrhoea* L., it was recommended to reject spraying in half of the areas, where it was previously planned because of the pests density at the level above the threshold of damage. These areas equalled 25000 hectares. The following observations showed the development of natural diseases in those locations which cause caterpillar death without damaging the trees. The rejection of irrational measures let to the preservation of the useful fauna and to the stabilization of the natural biocoenosis. Such rational planning of protective measures decreases significantly the danger to contaminate the environment with harmful residues of

chemical pesticides. It is of great importance that, in focus of the complex of entomopathogenic microorganisms, limitations of insecticide treatments create conditions for successful completion of the development of disease agents in the insects. Also, entomopathogens could form dormant stages persistent to any effects from outside and able to infect the next generations of the pest.

Numerous investigations into the usage of entomopathogens with selective action, i.e. nuclear polyhedrosis virus, granulosis virus, entomophtorous fungi, some species of non-sporeforming bacteria genus *Pseudomonas*, against lepidopterous forest pests showed the possibility to reserve useful compounds of the biocoenosis, including entomopathogens and entomophages. At the Ukraine the developed methods are used with success. Annually the measures on preserving the useful compounds of the biocoenosis, including natural pathogens and entomophages, are carried out in an area of 3 million hectares.

So, the experience of discovering, checking and preserving of entomopathogens in the forest biocoenosis provides the possibility of rational treatments with chemical and biological insecticides. It leads to a minimization of damage to useful compounds of the biocoenosis. A haematological method, based on histological and microbiological characteristics of haemolymph, as integral index of the physiological state of pest population, should be used in the planning of forest protection measures.

DEBILITATING EFFECTS ON SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEMENS), CAUSED BY TREATMENTS WITH SUBLETHAL DOSES OF *BACILLUS THURINGIENSIS VAR. KURSTAKI*.

A.F. Pedersen[1] and K. van Frankenhuyzen[2]

(1) Department of Ecology and Molecular Biology, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Copenhagen, Denmark.
(2) Forest Pest Management Institute, 1219 Queen St. E., P.O.Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7.

Laboratory experiments were conducted in order to investigate whether or not sublethal doses of *Bacillus thuringiensis* (Bt.) have any debilitating effects on the fitness of survivors of eastern spruce budworm larvae, *Choristoneura fumiferana* Clemens (Lepidoptera: *Tortricidae*). The effects on larval development time, pupal weight and fecundity were investigated. Fourth-, fifth-, and sixth-instar larvae were exposed for 72 h to sublethal doses of Bt. var. *kurstaki* HD-1-S-1980 incorporated into artificial diet. Fourth instars were significantly more susceptible than older instars. Exposure to Bt significantly increased larval development time by 22-37%, and reduced pupal weight by 17-28%. There was no significant effect on fecundity in spite the reduction in pupal weights and the high correlation between pupal weight and fecundity. However, treated females tended to lay fewer eggs and a higher proportion of infertile eggs.

INTRODUCTION

The spruce budworm, *Choristoneura fumiferana* Clemens, is a major defoliator of conifers in the boreal forests of North America. Commercial insecticides based on *Bacillus thuringiensis* (Bt) are widely used against this pest. Despite its high target specificity, extensive use of Bt. could still have an impact on nontarget organisms, such as soil microflora, nematodes and earthworms (reviewed by Addison, 1993), and forest Lepidoptera (Miller, 1992). It is thus important to attain spruce budworm control objectives while minimizing the use of Bt.

Recent progress has indicated the possibility of developing a population management strategy by integrating the present knowledge of the population biology of the spruce budworm with that of the microbial control agent. We postulate that a properly-timed treatment with Bt. will result in both protection of current year's foliage and longer term population suppression by enhancing natural mortality factors while debilitating fitness.

Because it is specific, Bt. is compatible with the action of naturally occurring parasitoids. Application of Bt during late larval stages enhanced conservation of the hymenopteran parasite *Apanteles fumiferanae* Vier by differentially killing nonparasitized larvae (Nealis & van Frankenhuyzen, 1990; Nealis *et al.*, 1992; Cadogan *et al.*, 1995). Exposure during late larval stages could also enhance the expression of sublethal effects. Ingestion of a sublethal dose causes temporary cessation of larval feeding, delayed larval development, and reduced pupal weight and fecundity (Morris, 1976; Morris, 1977; Smirnoff, 1983; Fast & Régnière, 1984; Alford & Holmes, 1986; van Frankenhuyzen & Nystrom, 1987; Ramachandran *et al.*, 1993). Such sublethal effects have the protential of influencing generation survival by debilitating population fitness.

The purpose of this study was to investigate if the expression of sublethal effects can be maximized by manipulating the timing of spray application. We exposed three instars to six doses of Bt. and investigated the effects on larval development time, pupal weight and fecundity of the survivors.

MATERIALS AND METHODS

Diet bioassays were conducted with early 4th-, 5th- and 6th-instar larvae from the laboratory stock maintained at the Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada. The larvae were exposed for 72 hrs to *Bacillus thuringiensis* var. *kurstaki* HD-1-S-1980 (16,000 IU/mg powder) incorporated into artificial diet. Six concentrations between 31.25 and 1000 IU/ml diet were used, based on preliminary experiments. Ten groups of five larvae per 25 ml-cup were used per dose level, including the control (no Bt). Mortality was recorded 5 days after transfer from diet containing Bt. Development time from emergence of 2nd-instar larvae to pupation was recorded for each larva and one-day-old pupae were weighed to the nearest 0.1 mg.

Each newly-emerged female moth was mated with a one-day-old male from the same treatment and left for 15 days in an oviposition cage containing a branch of balsam fir (*Abies balsamea* (L.) Mill.) for egg deposition. A new branch was provided every 5 days. The total number of eggs laid (actual fecundity) and the proportion that displayed the black headcapsules of developing larvae within 8 days at 25 °C (fertility) were recorded for each female. Females that only laid infertile eggs or none at all were dissected to determine the presence of spermatophores.

Treatment effects on development time, pupal weight and fecundity were determined by analysis of variance (SAS: ANOVA or GLM), assuming that these variables were normal distributed. The Bonferonni (Dunn) T-test was used to separate treatment means.

RESULTS AND DISCUSSION

Despite the preliminary experiments, the selected dose levels were too high. The higher than expected mortality resulted in a small number of pupae and moths in some treatments, particularly at the higher dose levels and in the 4th-instar treatment. Because there were no significant differences between dose levels in larval development time, pupal weight or fecundity, the six dose levels were pooled to contrast treated versus control within each instar.

Larval development time

There was a significant effect of instar, treatment, and sex on larval development time with a significant interaction between instar and treatment (Three-way ANOVA, Table 1). On average, exposure to Bt. increased the mean larval development time from 22% (4 days) in the sixth-instar treatment to 35% (7 days) in the fourth-instar.

Table 1. Development time (days) for spruce budworm larvae after exposure to sublethal doses (31.25 -1 000 IU/ml diet) of *Bacillus thuringiensis* var. *kurstaki* HD-1-S-1980 in their 4th, 5th or 6th stadium. Different letters indicate a significant effect at the 5% level (One-way ANOVA within instar and sex).

		4th instar		5th instar		6th instar	
Treatment		♀	♂	♀	♂	♀	♂
Control	n	22	21	22	16	20	25
	mean	21.3 a	19.6 a	21.6 a	20.3 a	18.8 a	17.8 a
	SD	3.6	2.1	1.9	1.6	0.8	1.1
Bt.	n	25	34	51	45	53	91
	mean	28.6 b	26.8 b	27.7 b	26.4 b	22.9 b	22.0 b
	SD	5.7	4.8	4.3	4.6	2.6	2.6

Pupal weight

There was a significant effect of instar, sex and dose on pupal weight with a significant interaction between instar and dose and between instar and sex (Three-way ANOVA, Table 2). Mean pupal weights decreased by 17-28%.

Table 2. Pupal weight (mg) of spruce budworm after exposure to sublethal doses of *Bacillus thuringiensis* var. *kurstaki* HD-1-S-1980 in their 4th, 5th or 6th stadium. Different letters indicate a significant difference at the 5% level (One-way ANOVA within instar and sex).

		4th instar		5th instar		6th instar	
Treatment		♀	♂	♀	♂	♀	♂
Control	n	22	21	22	16	20	25
	mean	108.8 a	82.3 a	99.4 a	77.7 a	102.0 a	75.5 a
	SD	29.4	24.7	30.8	15.6	20.6	15.0
Bt.	n	25	34	51	45	53	91
	mean	114.7 a	65.3 b	80.1 b	58.0 b	84.7 b	54.7 b
	SD	26.4	15.7	24.1	12.9	22.6	15.0

Fourth-instar females did not show decreased pupal weight in response to Bt exposure. This could be an artefact, due to selection of more vigorous individuals as a result of the higher mortality of 4th-instar larvae. The group of 4th-instar females consisted of only about half or even less of the number of individuals in the 5th- and 6th-instar groups. The few 4th-instars that survived the Bt exposure might have represented the most vigorous larvae. It could also be that these females, which also had the longest mean development time, had sufficient time to recover and regain normal pupal weight. In addition, the larvae treated in their 4th stadium were transferred to fresh diet and their numbers per cup were thus reduced earlier than for the larvae treated in their 5th and 6th stadia, thus introducing a bias towards more favourable growing conditions.

Fecundity

Since a significant difference between control and treated individuals was found for development time and pupal weight, one would expect an effect on fecundity as well. We found indeed a highly significant positive correlation between pupal weight and fecundity with correlation coefficients (R^2) of 0.73 and 0.66 for control and treated groups, respectively. However, the two correlation lines were nearly identical suggesting that Bt. exposure affected fecundity only through its effect on pupal weight. This was confirmed by an analysis of covariance, which indicated a significant effect of instar and of pupal weight as coviariate with no treatment effect or instar-treatment interaction. Although there was no significant difference in fecundity between treated and untreated females (Table 3), the fecundity of females treated as 5th or 6th instars tended to be lower. No tendency towards decreased fecundity was observed in females treated as 4th instars, suggesting that these were able to fully recover from the sublethal Bt. dose.

Table 3. Fecundity (eggs/female) of spruce budworm moths, *Choristoneura fumiferana*, after exposure to sublethal doses of *Bacillus thuringiensis* var. *kurstaki* HD-1-S-1980 in their 4th, 5th or 6th stadium (One-way ANOVA within instar).

Treatment		4th instar	5th instar	6th instar
Control	n	16	15	11
	mean	225.0 a	214.1 a	181.9 a
	SD	88.8	69.4	63.7
Bt.	n	18	39	32
	mean	235.4 a	174.3 a	152.5 a
	SD	71.1	75.2	59.8

The lack of a significant difference in fecundity between treated and untreated females despite the observed reduction in pupal weight and positive correlation of weight with fecundity could be due to high variability of the fecundity estimates and the relatively low sample sizes.

Females that did not lay any eggs or laid only infertile eggs were not included in the anlysis because they were evenly distributed over the treated and control groups. Infertile females did show a remarkable preponderance in the sixth-instar treatment (more than 50% of the infertile females were treated as 6th-instar). This might be due to the fact that pupae from the 6th-instar exposure were kept longer at a low temperature (13°C) than pupae from the 4th- or 5th-instar exposure. All but one of the 48 infertile females contained between 1 and 3 spermatophores, indicating that some couples had mated more than once. The second and third spermatophores were smaller than the first, and in some females the spermatophores had not been properly inserted, and were hanging out of the genital organs.

The difference between the total number of eggs laid and number of fertile eggs was submitted to an analysis of variance. There was no significant difference between treated and control groups. However there was a tendency for treated females to lay more infertile eggs than control females. For example, in the 5th-instar controls the mean number of infertile eggs made up 4.3% of the mean fecundity, whereas in treated females it was 9.5%.

CONCLUSION

Both larval development time and pupal weight were significantly altered after treatment with sublethal doses of Bt. Only females treated in their 4th stadium were able to attain normal pupal weight. There was no significant effect on fecundity in spite of reduced pupal weights and the high correlation between pupal weight and fecundity. However, treated females tended to lay fewer eggs and a higher proportion of infertile eggs. Further experiments using a larger number of larvae need to be conducted in order to shed more light on possible carry-over effects on the budworm's reproductive potential.

The effect of Bt. exposure on pupal weight and larval development time depended on the instar that was exposed, as indicated by significant treatment-instar interactions. However, the comparison between instars was confounded by differences in rearing conditions and a possible bias in selection for more tolerant individuals in the early-instar treatments. Standardization of rearing conditions for larvae in the various instar treatments together with a technique that permits quantitative and unbiased dosing of the various instars may yield more definitive data on the effect of sublethal Bt. doses on spruce budworm fitness.

In addition to the debilitating factors that have been investigated in this experiment other factors should also be studied in order to better understand the interactions between Bt. and its target insects, such as moth emergence, female longevity, egg weight and the emergence and viability of subsequent generations. Such studies would help lead to the most effective use of this biological pest contol agent, and consequently minimize its effects on nontarget organisms.

REFERENCES

Addison,J.A. 1993. Persistence and nontarget effects of *Bacillus thuringiensis* in soil: a review. Can. J. For. Res. 23: 2329-2342.

Alford,A.R. & Holmes,J.A. 1986. Sublethal effects of carbaryl, aminocarb, fenitrothion, and *Bacillus thuringiensis* on the development and fecundity of the spruce budworm (Lepidoptera: Tortricidae). J. Econ. Entomol. 79: 31-34.

Cadogan,B.L.; Nealis,V.G. & van Frankenhuyzen,K. 1995. Control of spruce budworm (Lepidoptera: Tortricidae) with *Bacillus thuringiensis* applications timed to conserve a larval parasitoid. Crop protection. 14: 31-36.

Fast,P.G. & Régnière,J. 1984. Effect of exposure time to *Bacillus thuringiensis* on mortality and recovery of the spruce budworm (Lepidoptera: Tortricidae). Can. Entomol. 116: 123-130.

Miller,J.G. 1992. Effects of a microbial insecticide, *Bacillus thuringiensis kurstaki,* on nontarget Lepidoptera in a spruce budworm infested forest. J. of Res. on the Lepidoptera. 29: 267-276.

Morris,O.N. 1976. A 2-year study of the efficacy of *Bacillus thuringiensis*-chitinase combinations in spruce budworm (*Choristoneura fumiferana*) control. Can. Entomol. 108: 225-233.

Morris,O.N. 1977. Long term study of the effectiveness of aerial application of *Bacillus thuringiensis* - acephate combinations against the spruce budworm, *Choristoneura fumiferana*

(Lepidoptera: Tortricidae). Can. Entomol. 109: 1239-1248.

Nealis,V. & van Frankenhuyzen,K. 1990. Interactions between *Bacillus thuringiensis* Berliner and *Apanteles fumiferanae* Vier. (Hymenoptera: Braconidae), a parasitoid of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 122: 585-594.

Nealis,V.G.; van Frankenhuyzen,K. & Cadogan,B.L. 1992. Conservation of spruce budworm parasitoids following application of *Bacillus thuringiensis* var *kurstaki* berliner. Can. Entomol. 124: 1085-1092.

Ramachandran,R.; Raffa,K.F.; Miller,M.J.; Ellis,D.D. & McCown,B.H. 1993. Behavioral responses and sublethal effects of spruce budworm (Lepidoptera: Tortricidae) and fall webworm (Lepidoptera: Arctiidae) larvae to *Bacillus thuringiensis* Cry1A(a) toxin in diet. Environ. Entomol. 22: 197-211.

Smirnoff,W.A. 1983. Residual effects of *Bacillus thuringiensis* and chemical insecticide treatments on the spruce budworm (*Choristoneura fumiferana* Clemens). Crop prot. 2: 225-230.

van Frankenhuyzen,K. & Nystrom,C.W. 1987. Effect of temperature on mortality and recovery of spruce budworm (Lepidoptera: Tortricidae) exposed to *Bacillus thuringiensis* Berliner. Can. Entomol. 119: 941-954.

THE INSECTICIDAL ACTIVITY OF SOME *BACILLUS THURINGIENSIS* STRAINS AGAINST FOREST *LEPIDOPTERA* LARVAE AT DIFFERENT TEMPERATURES

A. Sierpińska

Department of Forest Protection, Forest Research Institute
Bitwy Warszawskiej 1920 r. No. 3, 00-973 Warszawa, Poland

Summary

The study was carried out to estimate the insecticidal activity of a few *B. thuringiensis* strains (subspecies: *galleriae, kurstaki, sotto-dendrolimus and thuringiensis*) against two economically important forest defoliators: - *Dendrolimus pini* L. (*Lep.: Lasiocampidae*) and *Operophtera brumata* L. (*Lep.: Geometridae*), pine and oak pests respectively. The insecticidal activity of *B. thuringiensis* strains against both insect species was checked at two temperatures varied by 10°C. Measured responses of larvae to *B. thuringiensis* treatment were percentage mortality and reduction in frass weight. Median lethal time (LT_{50}) and correlation coefficients of mortality and frass production were calculated to point out the Bt strain most promising in control of the pests.
The sequence of tested *B. thuringiensis* strains by their insecticidal activity was as follows: *sotto-dendrolimus, kurstaki, thuringiensis, galleriae* for the *D. pini* larvae and *thuringiensis, sotto-dendrolimus, kurstaki, galleriae* for the *O. brumata* larvae. Temperature had an evident influence on both mortality and frass production.

Introduction

Until 1994, the use of the *B. thuringiensis* insecticides in the control of forest defoliators in Poland was not a common practice. Since that year *B. thuringiensis* Berliner subsp. *kurstaki* has become an agent used in the protection of forests against *Lymantria monacha* L. over large areas. Little is known about the insecticidal activity of *B. thuringiensis* subsp. *kurstaki* and other strains against *Lepidoptera* pests defoliating Polish forests and about an influence of environmental factors like temperature on the activity. The present study has been carried out to screen two economically important forest defoliating *Lepidoptera* for their susceptibility to some B. thuringiensis strains. *D. pini* L. and *O. brumata* L., pine and oak defoliators respectively, have been choosen as test insects. Reasons of the choice were also differences in the biology of the pests - various kinds of temperature - dependent responses could be expected.

Materials

The larvae of both species were taken from the forest, i. e. from a vital population of outbreak stage: the third instar of *D. pini* from the Miedzychod Forest District and the last instar of *O. brumata* from the Nowe Ramuki Forest District.
Bt strains: *galleriae* (# 159), *kurstaki* HD-1-S-1980 (a standard strain from USDA collection), *kurstaki* (re-isolated from Foray 48B, also HD-1), *sotto-dendrolimus* (# 565) and *thuringiensis* (# 058).

Methods

Bt powders and suspensions. All Bt strains except HD-1-S-1980 were cultivated at 30°C on nutrient agar. After one week, when spores amounted to above 90%, spore-toxin complexes were harvested and lyophilized. Powders of Bt were suspended in a saline buffer (0.85 % NaCl, 0.60 % K_2HPO_4 and 0.30 % KH_2PO_4) and sonicated. Tween 80 was added to a concentration 0.02 %.
Bioassays. Pine and/or oak twiggs, 30 cm long, were dipped in suspensions of Bt powders and allowed to dry. Then bunches consisting of 3 twiggs were formed, put into water and covered with glass pipes. Fifteen larvae were set down on one bunch.
For the testing of one Bt strain at one concentration and at one temperature 3 replications (3 bunches) were used. Assays for one insect species (5 Bt strains at 2 concentrations, at 2 temperatures, at 3 replications) ran simultanously. At the same time control larvae were reared on foliage dipped in a saline buffer with Tween 80. Assays for both insect species were repeated 3 times. Infected and control larvae were reared at 15 and 25°C (*D. pini*) or 12 and 22°C (*O. brumata*), 70 - 75 % RH and fotoperiod L:D-16:8.
During 8 (*O. brumata*) or 11 days (*D. pini*) of tests mortality of the larvae was checked everyday or every 2 days. To detect differences in the insecticidal activity of strains, data were analysed with the POLO-PC probit program (Russel at al., 1977; Robertson at al., 1992; LeOra Software, 1994) and LT_{50}'s were calculated. To check the significance of differences in LT_{50}'s a one-way analysis of variance (STATGRAPHICS Plus V.6.1, Manugistics, Inc., 1992) was used.
Frass was collected every 2 days, dried at 100°C and weighed.
To find the degree of correlation between mortality and frass production correlation coefficients were calculated.

Results

LT_{50} values. According to LT_{50}'s of tested Bt strains some differences in their insecticidal activity at all temperatures and between the insecticidal activities of one strain at different temperatures were found.

B. thuringiensis subsp. *galleriae* was not active against either insects and did not influenced the frass production.

Tab. 1. LT_{50}'s and slopes of the *D. pini* larvae treated with Bt strains at a concentration 50 µg/ml and reared at 15 and 25°C.
* LT_{50} of *D. pini* treated with strains *thu*, *kur* and HD and reared at 15°C was not estimated because of very low mortality of the larvae.

Temperature [°C]	Bt strain	count	LT_{50}	95 % Tukey HDS intervals for mean lower	upper	Slopes ± SE
15	den	3	15.58	15.04	16.07	4.10 ± 0.56
15	kur (re-isol.)*					
15	HD-1-S-1980*					
25	den	3	3.61	1.60	5.61	1.64 ± 0.28
25	kur (re-isol.)*					
25	thu*					
25	HD-1-S-1980	3	4.04	2.00	6.01	1.43 ± 0.27

The most active against the *D. pini* larvae was *B. thuringiensis* subsp. *sotto - dendrolimus* (Tab. 1).

Tab. 2. LT_{50}'s and slopes of the *O. brumata* larvae treated with Bt strains at concentration 50 µg/ml and reared at 12 and 22°C.

Temperature [°C]	Bt strain	count	LT_{50}	95 % Tukey HDS intervals for mean lower	upper	Slopes ± SE
12	den*	3				
12	kur (re-isol.)*					
12	thu	3	7.27	6.73	7.82	4.07 ± 0.80
12	HD-1-S-1980*					
22	den	3	5.13	5.36	6.10	5.53 ± 1.15
22	kur (re-isol.)	3	6.40	6.03	6.77	6.87 ± 1.15
22	thu	3	3.99	3.62	4.36	4.15 ± 1.06
22	HD-1-S-1980	3	3.35	2.98	3.72	2.93 ± 0.49

The highest insecticidal activity against the *O. brumata* larvae had *B.thuringiensis* subsp. *thuringiensis* (Tab. 2).

Frass production. Decrease of temperature by 10°C diminished by one third the frass production by the *D. pini* control larvae and 4.6 times the frass production by the *O. brumata* larvae. The inhibition of frass production of both species larvae at lower and higher temperatures occurrying after the treatment with Bt was evident.

The strongest inhibition in the *D. pini* larvae frass production was observed after the treatment with *B. thuringiensis* subsp. *sotto - dendrolimus*. In the case, the frass production was reduced

by half at higher temperature. After the treatment with *B. thuringiensis* subsp. *kurstaki* and *thuringiensis,* frass production was respectively 5.5 and 4.3 times more intensive at higher temperature.

The strongest inhibition in the *O. brumata* larvae frass production not occurred after the treatment with *B. thuringiensis* subsp. *thuringiensis,* the most active strain, but after the treatment with *B. thuringiensis* subsp. *kurstaki*. The *O. brumata* larvae treated with all Bt strains produced frass more intensive at higher temperature.

Correlation between mortality and frass production. Strong negative correlation for the *D. pini* larvae was only found when they were reared at 25°C after treatment with *B. thuringiensis* subsp. *sotto-dendrolimus* at both tested concentrations, *B. thuringiensis* subsp. *kurstaki* (re-isol.) and *kurstaki* HD-1-S-1980 at 500 µg/ml. Strong negative correlation for the *O. brumata* larvae was only found when they were treated with *B. thuringiensis* subsp. *thuringiensis* at both concentrations and reared at 12 and 22°C and *B. thuringiensis* subsp. *kurstaki* (re-isol.) at higher concentration and reared at higher temperature.

Conclusions

The sequence of tested Bt strains by their insecticidal activity was as follows: *sotto - dendrolimus, kurstaki, thuringiensis, galleriae* for the *D. pini* larvae and *thuringiensis, sotto - dendrolimus, kurstaki, galleriae* for the *O. brumata* larvae.

Temperature had an evident influence on both mortality and frass production.

A strong negative correlation between frass production and mortality of both species larvae was only found when larvae were treated with most active Bt strains and less active - at higher concentration and temperature.

The most promising in the control of *D. pini* seems to be *B. thuringiensis* subsp. *sotto - dendrolimus* and in the control of *O. brumata - B. thuringiensis* subsp. *thuringiensis.*

Acknowledgments

I thank Mr A. Temple Bowen, Jr., Novo Nordisk for his support in the preparing of the presentation.

References

Robertson, J.L. & Preisler, H., 1992. Pesticide Bioassays with Arthropods.
CRC Press.

Russell, R.M., Robertson, J.L. & Savin, S.E., 1977. POLO: A new computer program for probit analysis.
Bull. Entomol. Soc. Am. **23**: 209-213.

3. Entomopathogenic Nematodes & their Symbiotic Bacteria

Biology of entomopathogenic nematode symbionts : Recent advances

Noël Boemare, Alain Givaudan, Jacques-Olivier Thaler
and Marie-Hélène Boyer-Giglio
Laboratoire de Pathologie comparée, Université Montpellier II,
URA INRA-CNRS n° 1184
34095 MONTPELLIER CEDEX 5, FRANCE

The genus *Xenorhabdus*, member of the *Enterobacteriaceae*, consists of the specific bacterial symbionts of the entomopathogenic nematodes *Steinernematidae* (Thomas and Poinar, 1979), and was separated from the genus *Photorhabdus* which consists almost entirely of symbionts of the entomopathogenic nematodes *Heterorhabditidae* (Boemare *et al.*, 1993). The nematodes provide protection and transport for their bacterial symbionts carrying them monoxenically in a special vesicle of infective stage (L3 juveniles) of *Steinernematidae* or throughout the whole intestine of *Heterorhabditidae.* They release them into the hemocoel of the insect prey, inducing a lethal septiceamia. The bacterial symbionts contribute to the symbiotic relationship by establishing and maintaining suitable conditions for the nematode reproduction (Akhurst and Boemare, 1990)

Two phase variants occur spontaneously which have been characterized by a series of different physiological properties (Boemare and Akhurst, 1988). Phase I variants of *Xenorhabdus* adsorb dyes on agar plates, produce phospholipase (lecithinase), and have protoplasmic paracrystalline inclusions, whereas these properties are either apparently absent or greatly reduced in phase II variants. Phase I variants produce chemical, agar diffusible antibiotics while phase II variants produce no such compounds (Akhurst, 1982). It is clearly demonstrated that phase change occurs during the stationary period of growth and that the extent of phase variation is highly variable and not well controlled (Boemare and Akhurst, 1990). Phase I variants has been reported to provide a better support for the nematode growth than phase II. Infective juvenile nematodes resulting from natural parasitism in insects or from *in vitro* cultures in the presence of both variants contain only phase I variants (Akhurst and Boemare, 1990).

A lysogeny concomitant with a bacteriocinogeny was demonstrated in *Xenorhabdus* spp. (Boemare *et al.*, 1992). Entire phage particles were detected in few quantities (5-10%) with a larger amount of bacteriocins and phage heads in cultures of both phases after induction by mitomycin C or heat treatments. Baghdiguian *et al.* (1993) have shown that *Xenorhabdus* bacteriocins have a similar structure as rigid bacteriophage tails, that is being made up of a contractile sheath, a core, and a baseplate with six caudal fibers. Similar features of bacteriocinogenesis were shown in *Photorhabdus* and *Xenorhabdus* cells. Boemare *et al.* (1992) showed two distinctive

types of antimicrobial activity in *X. nematophilus*. The broad spectrum activity of antimicrobial molecules (Akhurst, 1982) produced by phase I variants was confirmed, as well as a bactericidal activity from both variant bacteriocins against a much more limited range of bacteria, mainly those closely related to *X. nematophilus*. Basically both variants of other *Xenorhabdus* spp. were sensitive to *X. nematophilus* bacteriocins.

This report summarizes some recent works published the first half-year of 1995 about the knowledge of the entomopathogenic symbionts biology.

Swarming Behavior

It was shown that *Xenorhabdus* phase I variants displayed a swarming motility when grown on media containing 0.6 to 1.2% agar (Givaudan *et al.*, 1995). Whereas most of the phase I variants from different *Xenorhabdus* spp. were able to undergo cycle of rapid and coordinately population migration over the surface, most of the phase II variants were unable to swarm and even to swim in semi-solid agar, particularly in *X. nematophilus*. Optical and electron microscopic observations showed non-motile cells with phases II of *X. nematophilus* F1 which lost their flagella. Flagellar filaments from *X. nematophilus* strain F1 phase I variants were purified, and the molecular mass of the flagellar structural subunit was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to be 36.5 kDa. Flagellin from cellular extracts or culture medium of phase II was undetectable with antiserum against the denatured flagellin using immunoblotting analysis. The most important finding of this work is the demonstration that phase II variants of *X. nematophilus* F1 are not only unable to swarm, but phase II cells are also not flagellated. The importance of a such difference of motility between both phases is a feature of adaptation of these bacteria to the insect prey and the nematode host.

Surface components and cellular appendages

Cellular appendages occur only in phase I variants of *Xenorhabdus*. They were particularly studied in *X. nematophilus* (Givaudan *et al.*, 1995). The lack of flagella in phase II cells is due to a defect during flagellin synthesis. The genes involved in the synthesis of flagella were cloned and sequenced. They shared similarities with the gene coding for the flagellin, *Fli*C, and the gene coding for the capping protein, *Fli*D, of other Enterobacteriaceae (Givaudan *et al.*, unpublished data). The level of flagellar regulation is currently investigated.

X. nematophilus strain express fimbriae when the bacteria were grown on a solid media with 15% agar (Moureaux *et al.*, 1995). They were rigid, with a diameter of 6.4 ± 0.3 nm and were composed of 16-kDa pilin subunits. The latter were synthesized and assembled during the first 24 h of growth. NH2-terminal sequencing of the pilin revealed homology with other Enterobacteriaceae pilin (Moureaux *et al.*, unpublished

data). Phase II variants of *X. nematophilus* did not possess fimbriae and apparently did not synthesize pilin. Study of the level of pilin regulation is also in progress.

Phase I variants of *X. nematophilus* have and/or their purified fimbriae have an agglutinating activity with sheep, rabbit and human erythrocytes and with hemocytes of the insect *Galleria mellonella* (Moureaux *et al.*, 1995). The agglutination was mannose-resistant and was inhibited by porcine gastric mucin and N-acetyl-lactosamine. This last sugar seems to be a specific inhibitor of hemagglutination by *X. nematophilus*. These agglutinating properties are the first demonstration of the adhesive properties of *Xenorhabdus*. Fimbriae have been shown to be an important virulence factor in enterotoxigenic or uropathogenic vertebrate Enterobacteriaceae by enhancing colonization of epithelial cells.

With strains of *Photorhabdus luminescens*, Gerritsen *et al.* (1995) prepared polyclonal antisera useful to distinguish strains and phase variants by different diffusion patterns. The specificity of each phase variant antiserum was enhanced by cross-absorption allowing a serotyping of the variants. It is likely that these polyclonal antisera recognized similar appendages as the previous fimbriae and flagella of *Xenorhabdus* phase I variants identified also by antisera which were prepared from the purified proteins. To probe a such hypothesis, purification of pilin and flagellin of *Photorhabdus* should be undertaken.

Outer membrane proteins were examined in both variants of *Xenorhabdus nematophilus* (Leisman *et al.*, 1995). The amino acid composition of OpnP, the major porin protein in *Xenorhabdus nematophilus*, was very similar to those of the porins of *E. coli*. Three additional proteins, OpnA, OpnB, and OpnS, were induced during stationary period of growth. OpnA and OpnB were only produced in phase I variants. The authors suggested that the inefficient production of nematodes grown on phase II variants may be due , in part, to the absence of OpnB and other cell surface molecules that play a role in adhesion and colonization.

Bacteriocinogeny and lysogeny.

The rigid phage tail like particles have been evidenced to be different from the flexible tails of the entire phage particles accompanying the bacteriocinogeny. Xenorhabdicin, the phage tail-like bacteriocins of *Xenorhabdus nematophilus,* produced after mitomycin induction in *X. nematophilus* lysogenic strain F1 cultures, were separated by DEAE chromatography, examined by transmission electron microscopy, and characterized by SDS-PAGE (Thaler *et al.*, 1995). Electrophoresis of xenorhabdicin showed two major subunits of 43 kDa and 20 kDa corresponding to the sheath and the inner core, respectively. Bactericidal activity recorded against closely related bacterial species, and spontaneously produced by *X. nematophilus*, resides in the xenorhabdicin particles and is another antimicrobial barrier to save the symbiotic association.

Study of the ultrastructure and the replication of the phages is in progress (Boyer-Giglio & Fournier, unpublished data). Virions have an isometric head and a long non-contracted flexible tail different from the xenorhabdicins. Experiments have shown that the phage DNA is double-stranded, linear, with non-cohesive extremities. To increase the production of phage particles, in front of the xenorhabdicin production, mitomycin treatment must be applied only during a short period before re-incubation.

The knowledge of the biology of nematode entomopathogenic symbionts was increasing this last period and promises some interesting discoveries in the nearest future.

References

Akhurst, R.J. 1982. Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the families *Heterorhabditidae* and *Steinernematidae*. J. gen. Microbiol. 128: 3061-3065.

Akhurst, R.J., and N. Boemare. 1990. Biology and Taxonomy of *Xenorhabdus* . In : *Entomopathogenic nematodes in biological control*. R., Gaugler, H.K., Kaya, eds. CRC Press, Boca Raton, Fl., USA. pp.75-90.

Baghdiguian, S., M-H. Boyer-Giglio, J.-O. Thaler, G. Bonnot, and N. Boemare. 1993. Bacteriocino-genesis in cells of *Xenorhabdus nematophilus* and *Photorhabdus luminescens* : Enterobacteriaceae associated with entomophathogenic nematodes. Biol.Cell 79: 177-185.

Boemare, N. E., and R. J. Akhurst. 1988. Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (*Enterobacteriaceae*). J. Gen. Microbiol. 134:1835-1845.

Boemare, N. E., and R. J. Akhurst. 1990. Physiology of phase variation in *Xenorhabdus* spp., p. 208-212. *In* Society for Invertebrate Pathology (ed.), Vth international colloquium on invertebrate pathology and microbial control, Adelaide, Australia.

Boemare, N. E., R. J. Akhurst, and R. G. Mourant. 1993. DNA relatedness between *Xenorhabdus* spp. *(Enterobacteriaceae*), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen.nov. Int. J. Syst. Bacteriol. 43:249-255.

Boemare, N.E., M-H. Boyer-Giglio, J.-O. Thaler, R. Akhurst, and M. Brehélin. 1992. Lysogeny and bacteriocinogeny in *Xenorhabdus* spp., bacteria associated with entomopathogenic nematodes. Appl. Environ. Microbiol. 58 : 3032-3037

Gerritsen, L., J. Van der Wolf, J. Van Vuurde, R.-U. Ehlers, K. Krasomil-Osterfeld, and P. Smits. 1995. Polyclonal antisera to distinguish strains and form variants of *Photorhabdus* (*Xenorhabdus) luminescens*. Appl. Environ. Microbiol. 61: 284-289

Givaudan, A., S. Baghdiguian, A. Lanois, N. Boemare. 1995. Swarming and swimming changes concomitant with phase variation in *Xenorhabdus nematophilus*. Appl. Environ. Microbiol. 61: 1408-1413.

Leisman, G., J. Waukau, and S. Forst. 1995. Characterization and environmental regulation of outer membrane proteins in Xenorhabdus nematophilus. Appl. Environ. Microbiol. 61: 200-204.

Moureaux, N., T. Karjalainen, A. Givaudan, P. Bourlioux, and N. Boemare. 1995. Biochemical characterization and agglutinating properties of *Xenorhabdus nematophilus* F1 fimbriae. Appl. Environ. Microbiol. 61: 2707-2712.

Thaler, J.-O., S. Baghdiguian, and N. Boemare. 1995. Purification and characterization of nematophilicin, phage tail like bacteriocin, from the lysogenic strain F1 of *Xenorhabdus nematophilus*. Appl. Environ. Microbiol. 61: 2049-2052.

Thomas, G. M. , and G. O. Poinar. 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic and nematophilic bacteria of the family *Enterobacteriaceae*. Int. J. Syst. Bacteriol 29:352-360.

CONTROL OF SCARABAEID LARVAE IN GOLF COURSE TURF WITH THE ENTOMOPATHOGENIC NEMATODES *HETERORHABDITIS MEGIDIS* AND *H. BACTERIOPHORA*

R.-U. EHLERS, D. SULISTYANTO AND J. MARINI

Institute for Phytopathology, Dep. Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Klausdorfer Str. 28-36, 24223 Raisdorf, Germany

SUMMARY

Liquid culture produced entomopathogenic nematodes, *Heterorhabditis megidis* and *H. bacteriophora*, were applied in May and June 1994, respectively, at 0,5 and 1,5 Mio. dauer juveniles/m² on a golf course in Northern Germany. The grub density was recorded in June and August 1994 and in March 1995 on plots treated with *H. megidis* and in July, September 1994 and March 1995 on those treated with *H. bacteriophora*. A significant reduction of the grub density was recorded for both nematode species in 1994. Two grub species occurred, *Phyllopertha horticola* and a species of the genus *Aphodius*, probably *A. contaminatus*.

The results are summarized in Tab. 1. Control of *Aphodius* sp. 42 days after application of *H. megidis* reached 40 and 53% at low and high nematode density, respectively. 29 days after application of *H. bacteriophora* the total control of both species was 55 and 62%. Control of *Phyllopertha horticola* caused by *H. megidis* recorded in August reached 52% and 70%. In September the control due to *H. bacteriophora* was 65 and 83%. Turf damage caused by birds or mammals preying on the grubs was successfully prevented. In March 1995 only plots treated with *H. bacteriophora* showed a significant reduction of *Aphodius* sp., whereas on plots treated with *H. megidis* the nematodes had not persisted long enough to cause a significant reduction and damage was recorded. The overall higher control achieved with *H. bacteriophora* could possibly be a result of the introduction of this non-endemic species, which has only been recorded in Southern Germany.

Detailed information is given in Sulistyanto *et al.* (1995).

ACKNOWLEDGEMENTS

Thanks are due to the greenkeeper M. Paletta for the provision of test plots and spraying equipment, to Ecogen Bio Germany GmbH, Raisdorf for providing nematodes, to many collegues for help with sampling, to H. Vlug (IPO-DLO, Wageningen, The Netherlands) for providing unpublished data and to P. Hirschberger, Kiel, for the identification of *A. contaminatus*. The scholarship to DS by the Deutscher Akademischer Austauschdienst, to GM by 3A, Italy and the financial support for the co-operation with the IPO-DLO by the European Union COST Action 819 is gratefully acknowledged.

Tab. 1: Mean grub density (*Aphodius* sp. and *Phyllopertha horticola*) ± standard deviation per 400 cm² sample (5/plot) recorded at different dates (days) after application of liquid culture produced *Heterorhabditis megidis* or *H. bacteriophora* at 0,5 and 1,5 Mio. nematodes/m² on treated and control plots (*H. megidis* 3x8 plots, *H. bacteriophora* 3x15 plots). Means with the same letter are not significantly different (Tukey's Test, $P<0,05$).

H. megidis			
Days after application Date of sampling	42 16/6/94	111 24/8/94	309 10/3/95
Grub species	*Aphodius* sp.	*P. horticola*	*A. contaminatus*
0,5 Mio./m²	11,8a ±3,5	2,1a ±0,5	1,9a ± 0,7
1,5 Mio./m²	9,0a ±3,8	1,3b ±0,5	1,8a ± 1,3
Control	19,5b ±3,0	4,5c ±0,6	2,6a ± 2,1
H. bacteriophora			
Days after application Date of sampling	29 21/7/94	98 28/9/94	265 14/3/95
Grub species	*P. horticola* and *Aphodius* sp.	*P. horticola*	*A. contaminatus*
0,5 Mio./m²	5,0a ±2,6	1,4a ±0,4	3,5a ± 1,5
1,5 Mio./m²	4,1a ±3,2	0,7b ±0,2	3,0a ± 1,4
Control	10,9b ±2,3	4,1c ±0,7	9,6b ± 2,0

REFERENCE

Sulistyanto, D., Marini, G. & Ehlers, R.-U. (1995). Biological control of scarabaeid larvae in golf course turf with the entomopathogenic nematodes *Heterorhabditis megidis* and *H. bacteriophora*. Biocontrol Sci. & Technol. **5**, in press.

Preference of *Heterorhabditis megidis* for *Photorhabdus luminescens* (*Xenorhabdus luminescens*) strains and form variants.

L.J.M. Gerritsen and P.H. Smits.

Research Institute for Plant Protection (IPO-DLO), Binnenhaven 5, P.O.B. 9060, 6700 GW Wageningen, The Netherlands.

Summary To test the preference of nematodes on feeding on, and retention of symbiont stains and form variants a preference test was done with infective juveniles of *Heterorhabditis megidis* strains DH-SH1 (HSH) and NLH.E87.3 (HE) and there symbiotic bacteria *Photorhabdus luminescens* strains PSH and PE, respectively.

Both nematodes could multiply on their own primary form symbiont and on the primary form bacterium of the other nematode. In a mixture the nematodes did feed on both primary forms and did not prefer one strain to the other. Both nematodes could not reproduce on the secondary form of their own symbiont. HE could not reproduce on the secondary form PSH/2 but, in contrast, HSH could multiply on secondary form PE/2. This suggests that the negative effect a secondary form bacterium has on nematode reproduction is not a common factor in all secondary forms.

Both nematodes could multiply on a combination of primary and secondary form bacteria, but the nematodes preferred the primary form as a food source.

All nematodes were able to retain the bacteria they had been cultured on. HSH and HE cultured on a mixture of their primary and secondary form were able to take up both forms in their guts, although the infective juveniles contained more primary cells than secondary cells.

These results suggest that the nematodes prefer the primary form as a food source but the retention of bacteria is not as specific.

INTRODUCTION

Photorhabdus luminescens (Boemare *et al.* 1993) and *Xenorhabdus* spp. (Akhurst and Boemare, 1990), are insect pathogenic bacteria symbiotically associated with nematodes of the genera *Heterorhabditis* and *Steinernema*, respectively (Thomas & Poinar, 1979). The infective dauerjuvenile of the nematode carry the bacterial symbiont in the intestine (Bird and Akhurst, 1983; Endo and Nickle, 1991). The nematode penetrates an insect host, moves into the haemocoel, and releases the bacterium. The bacterium starts

multiplying and kills the host, helped by excretion products of the nematode that repress the immune system of the insect (Götz *et al.* 1981). *P. luminescens* further produces antibiotics to inhibit growth of other micro-organisms in the insect cadaver (Akhurst, 1982; Gerritsen *et al.*, 1992) and provides nutrients utilized by the nematodes (Poinar & Thomas, 1966).

The colony morphology and biochemical abilities of *Photorhabdus* and *Xenorhabdus* isolates are highly variable. Two extreme colony forms are characterized as phase 1 and phase 2 or, respectively, primary and secondary form (Akhurst, 1980; Akhurst and Boemare, 1990; Bleakley and Nealson, 1988; Boemare and Akhurst, 1988) but also several colony forms with intermediate properties have been described (Gerritsen *et al.*, 1992; Hurlbert, 1989).

Studies have shown significant difference in the ability of various strains of *P. luminescens* to support cultures of non-host *Heterorhabditis* spp. and in the ability of the nematode to retain the bacteria in the infective juveniles (Gerritsen and Smits, 1993; Gerritsen *et al.* 1996; Han *et al.*, 1990).

In this paper the preference of two *H. megidis* strains for feeding on, and retention of symbiont stains and form variants was studied.

MATERIALS AND METHODS

Nematode and bacterial isolates

Two *Heterorhabditis megidis* strains were used, strain NLH.E87.3 (HE) from The Netherlands and strain DH.SH1 (HSH) from Germany. The primary form bacteria, PSH/1 from HSH and PE/1 from HE, were isolated directly from infective stage nematodes, while the secondary form bacteria, PSH/2 from HSH and PE/2 from HE, were isolated after induction of the primary form under low osmotic conditions (Krasomil-Osterfeld, 1995). Bacteria were grown on nutrient agar (0.8% Lab Lemco Broth, Oxoid; 1.5% agar) and incubated in the dark at 25°C for 3 days.

Preference test

Bacteria were inoculated on lipid agar (16 g/l nutrient broth (Bacto), 5 g/l corn oil, 12 g/l agar (Wouts, 1981)) in a 5 cm Petri dish. One third of the plate was inoculated with one bacterium and an other third with another (or, as a control, the same) bacterium. Plates were incubated in the dark at 25°C. After one day 100 infective juveniles (monoxenically cultured, surface sterilized in 0.4 % Hyamine) of either HSH or HE were put on the remaining third of the agar plate. Plates were incubated in the dark at 25°C and nematode growth and reproduction was monitored. This test was done three times.

Uptake of bacteria by nematodes

Infective juveniles produced in the preference test were surface sterilized in 0.4 % Hyamine for 15 min., washed twice with sterile demineralized water and crushed in a Potter homogenizer. Of this suspension 50 µl was inoculated on MacConkey agar plates, and 50 µl of a 1:10 dilution of this suspension in demineralized water was inoculated on nutrient agar plates. This undiluted and 1:10 diluted nematode-bacterium suspension was used in indirect immunofluorescence cell-staining (IF). Indirect IF was performed according to Gerritsen *et al.* (1995) with four antisera specific for the four bacteria. The antisera are polyclonal, produced in rabbits against live, whole cells and cross absorbed to overcome cross reaction (Gerritsen *et al.* 1995).

Table 1. Production of infective juveniles of *Heterorhabditis* strains HSH and HE on different bacteria forms and combinations of bacteria. Total number of infective juveniles produced on one lipid agar plate (mean of three experiments).

nematode bacteria	HSH	HE
PSH/1	26,700	31,200
PSH/2	0	*900
PE/1	17,250	38,300
PE/2	9,500	0
PSH/1 + PE/1	25,400	34,950
PSH/2 + PE/2	0	*2,200
PSH/1 + PSH/2	7,900	10,950
PE/1 + PE/2	8,250	13,900

* result of one experiment, other experiment no infective juvenile production

RESULTS

Preference test

Table 1 shows the production of infective juveniles of HSH and HE on different bacteria combinations. Single bacterium: Both nematodes could multiply on their own primary form symbiont and on the primary form bacterium of the other nematode. Both nematodes could not reproduce on the secondary form of their own bacterium. Although some infective juveniles, inoculated on the secondary form, developed to hermaphrodites, only some

of these hermaphrodites produced eggs and all J1 hatched from these eggs died. HSH could multiply on secondary form PE/2, although the production of infective juveniles on these plates was less then on one of the primary forms. Only in the third experiment HE could multiply on secondary form PSH/2, in the other two experiments all nematodes died before they could produce a second generation. Combination of primary forms: Both nematodes multiplied very well on a combination of primary form bacteria and both bacterial lawns had been eaten at the end of the experiment. Combination of secondary forms: HE did multiply on both secondary forms in the first but not in the other two experiments. HSH could not multiply on a combination of secondary forms.

Primary + secondary form: Both nematodes could multiply on a combination of primary and secondary form bacteria. The production of infective juveniles on these plates was less then on plates with pure primary form (table 1). When put on a plate with primary and secondary from bacteria, most infective juveniles preferred the primary form bacteria. Some wandered through the secondary form bacterial lawn but settled in the primary form soon. Also HSH, which could reproduce on PE/2, preferred primary form PE/1 to secondary form PE/2 when there was choice. Second generation nematodes wandered through the whole plate but at the end of the experiment the primary form bacterial lawn had been eaten by the nematodes while most of the secondary form bacterial lawn was still there. Except for HSH which eat both PE/1 and PE/2 in the end.

Retention of bacteria by nematodes

All nematodes were able to take up the bacteria they had been cultured on. When cultured on one bacterium only, the infective juveniles carried this bacterium in their guts, in all cases. Even HSH infective juveniles cultured on PE/2 carried many bacteria in their guts.

When cultured on a mixture of bacteria, nematodes contained both bacteria in their guts. When cultured on both primary forms, HSH and HE contained both bacteria in there guts in equal amounts. Only in one experiment HE reproduced on a combination of PSH/2 and PE/2 and the infective juveniles produced on this plate contained both bacteria in equal amounts.

HSH and HE cultured on a mixture of their primary and secondary form were able to take up both forms in their guts. IF results showed that the nematodes contained more PSH/1, primary cells than PSH/2, secondary cells.

DISCUSSION

Heterorhabditis spp. need bacteria of the species *Photorhabdus luminescens* to provide them with essential nutrients. In contrast to *Steinernema* spp. *Heterorhabditis* spp. can not be cultured without symbiotic bacteria (Akhurst, 1986; Ehlers *et al.* 1990; Lunau *et al.*, 1993). Not every *P. luminescens* strain can be used as a food source for a *Heterorhabditis* spp.. Gerritsen and Smits (1993) and Han *et al.* (1990, 1991) showed that some

Heterorhabditis spp. are not able to reproduce on *P. luminescens* strains of other *Heterorhabditis* spp.. The results presented here show that *H. megidis* strains HSH and HE are able to reproduce on each others primary form symbiont. When the nematodes have a choice to feed on their own symbiont or on the symbiont of the other nematode they have no preference. They feed on both bacteria and also take up both bacteria in their guts.

Form variation in *P. luminescens* also has effect on nematode reproduction. It is often stated that the secondary form does not support growth and reproduction of nematodes as well as the primary form (Akhurst, 1980; Akhurst & Boemare, 1990; Ehlers *et al.* 1990). Results presented in this paper also show that both *H. megidis* strains are not able to reproduce on the secondary form of their own symbiont. On the other hand, *H. megidis* strain HSH was able to multiply on secondary form PE/2 of strain HE. This suggests that the negative effect a secondary form bacterium has on nematode reproduction is not a common factor in all secondary forms. Although the low support of nematode reproduction is mentioned as a common characteristic of all secondary forms (Akhurst and Boemare, 1990) the expression of this characteristic differs per secondary form, since HSH can reproduce on PE/2 but not on PSH/2. Also the sensitivity of the nematode differs per strain since HSH can reproduce on PE/2 but HE can not. HE could reproduce on PSH/2 only in one experiment in very low amounts.

All nematodes retain the bacteria they have been cultured on. When nematodes are cultured on a mixture of bacteria and both bacteria are a good food source then the nematodes carry both bacteria in equal amounts and there is no preference for there own symbiont. When cultured on both primary and secondary form the infective juveniles carry both bacteria but not in equal amounts, the nematodes retain more primary than secondary form bacteria. Akhurst (1980) states that *Steinernema* and *Heterorhabditis* spp. preferentially take up the primary form when cultured on a mixture of primary and secondary form, with the exception of one *H. heliothidis* strain. The results presented here show that although the nematodes carry more primary form cells, they also carry secondary cells. It is possible that the nematodes do not distinguish between primary and secondary cells when they take up cells. Because the nematodes prefer the primary form as a food source they get in contact with primary form cells more and therefore carry more of these cells. HSH cultured on PE/2 did carry this secondary form in as large amounts as a primary form. This suggests that there is no preference for retaining a primary form, and all bacteria are retained that the nematode has been feeding on.

References

AKHURST, R. J. 1980. Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *J. Gen. Microbiol.*, 121 : 303-309.

AKHURST, R. J. 1982. Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. *J. Gen. Microbiol.*, 128 : 3061-3065.

AKHURST. R. J. 1986. *Xenorhabdus nematophilus* supsp. *poinarii*: its interaction with insect pathogenic nematodes. *System. Appl. Microbiol.*, 8 : 142-147

AKHURST, R. J. & BOEMARE, N. E. 1990. Biology and taxonomy of *Xenorhabdus*. *In :* Gaugler R. & Kaya, H. K. (Eds) *Entomopathogenic nematodes in biological control*. Boca Raton, FL, CRC Press : 75-90.

BIRD, A. F. & AKHURST, R. J. 1983. The nature of the intestinal vesicle in nematodes of the family Steinernematidae. *Int. J. Parasit.*, 13 : 599-606.

BLEAKLEY, B. & NEALSON, H. K. 1988 Characterization of primary and secondary forms of *Xenorhabdus luminescens* strain Hm. *FEMS Microbiol. Ecol.* 53 : 241-250.

BOEMARE, N. E., & AKHURST, R. J. 1988. Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (Enterobacteriaceae). *J. Gen. Microbiol.*, 134 : 751-761.

BOEMARE, N. E., AKHURST, R. J. & MOURANT, R. G. 1993. DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. *Int. J. Syst. Bacteriol.*, 43 : 249-255.

ENDO, B. Y. & NICKLE, W.R. 1991. Ultrastrucutre of the intestinal epithelium, lumen and associated bacteria in *Heterorhabditis bacteriophora*. *J. Helminthol. Soc. Wash.*, 58 : 202-212.

EHLERS, R.-U., STOESSEL, S. & WYSS, U. 1990. The influence of the phase variants of *Xenorhabdus* spp. and *Escherichia coli* (Enterobacteriaceae) on the propagation of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*. *Rev. Nematol.*, 13 : 417-424.

GERRITSEN, L. J. M., DE RAAY, G. & SMITS, P. H. 1992. Characterization of form variants of *Xenorhabdus luminescens*. *Appl. Environ. Microbiol.*, 58 : 1975-1979.

GERRITSEN, L. J. M. & SMITS, P. H. 1993. Variation in pathogenicity of recombinations of *Heterorhabditis* and *Xenorhabdus luminescens* strains. *Fund. Appl. Nematol.*, 16 : 367-373.

GERRITSEN, L. J. M., VAN DER WOLF, J. M. VAN VUURDE, J. W. L., EHLERS, R.-U., KRASOMIL-OSTERFELD, K.C. & SMITS, P. H. 1995. Polyclonal antisera to distinguish strains and form variants of *Photorhabdus luminescens (Xenorhabdus luminescens)*. *Appl. Environ. Microbiol.*, 61 : 248-289.

GERRITSEN, L. J. M. & SMITS, P. H. 1996. Pathogenicity of new combinations of *Heterorhabditis* spp. and *Photorhabdus luminescens (Xenorhabdus luminescens)* against *Galleria mellonella* and *Tipula oleracea*. In prep.

GÖTZ, P., BOMAN, A. & BOMAN, N. 1981. Interaction between insect immunity and an insect pathogenic nematode with symbiotic bacteria. *Proc R. Soc. Lond. B Biol. Sci.*, 212 : 333-350.

HAN, R. C., WOUTS, W. M., & LI, L. Y. 1990. Development of *Heterorhabditis* spp. strains as characteristics of possible *Xenorhabdus luminescens* subspecies. *Revue Nématol.*, 13 : 411-415.

HAN, R. C., WOUTS, W. M., & LI, L. Y. 1991. Development and virulence of *Heterorhabditis* spp. strains associated with different *Xenorhabdus luminescens* isolates. *J. Invertebrate Pathol.*, 58 : 27-32.

HURLBERT, R. E., XU, J., & SMALL, C. L.. 1989 Colonial and cellular polymorphism in *Xenorhabdus luminescens*. *Appl. Environ. Microbiol.* 55 : 1136-1143.

KRASOMIL-OSTERFELD, K. C. 1995. Influence of osmolarity on phase shift in *Photorhabdus luminescens*. *Appl. Environ. Microbiol.*, 61 : in press.

LUNAU, S., STOESSEL, S., SCHMIDT-PEISKER, A. J., & EHLERS, R.-U. 1993. Establishment of monoxenic inocula for scaling up *in vitro* cultures of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp.. *Nematologica*, 39 : 385-399.

POINAR, G. O., & THOMAS, G. M. 1966. Significance of *Achromobacter nematophilus* (Achromobacteriaceae: Eubacteriales) in the development of the nematode, DD-136 (*Neoaplectana* sp.; Steinernematidae). *Parasitology*, 56 : 385-390.

THOMAS, G. M., & POINAR, G. O. 1979. *Xenorhabdus* gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae. *Int. J. Syst. Bacteriol.*, 29 : 352-360.

WOUTS, W. M., 1981. Mass production of the entomogenous nematodes *Heterorhabditis heliothidis* (Nematoda: Heterorhabditidae) on artificial media. *J. Nematol.*, 13 : 467-469.

GROWTH AND METABOLISM OF *PHOTORHABDUS LUMINESCENS* UNDER CONTINUOUS CULTURE CONDITIONS

C. MÄTJE[1], P.H. SMITS[2], L.J.M. GERRITSEN[2] AND R.-U.EHLERS[1]

(1) Institute for Phytopathology, Dep. Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Klausdorfer Str. 28-36, 24223 Raisdorf, Germany
(2) Research Institute for Plant Protection (IPO-DLO), Binnenhaven 12, P.O.B. 9060, 6700 Wageningen, The Netherlands

SUMMARY

Growth and metabolism of *Photorhabdus luminescens* were recorded under continuous liquid culture conditions in a chemostat. The bioluminescence activity and the production of antibiotics were lost when batch culture conditions were adjusted and the activities were restored after the addition of fresh medium. Independently of the various culture conditions cells could only be labelled with a primary form, but not with a secondary form specific antibody. Neither decreasing osmolarity nor changing growth conditions induced a phase shift to secondary form according to the results obtained with the antibodies.

INTRODUCTION

The entomopathogenic nematode *Heterorhabditis* sp. is produced together with its symbiont *Photorhabdus luminescens* in liquid culture bioreactors. The bacteria are the essential food source for the nematodes. They support nematode development and reproduction. *P. luminescens* produces phase variants, commonly described as primary and secondary form. The mechanism and function of the phase shift are still unknown. Along with the phase shift many characters change (e.g. bioluminescence activity, pigmentation, antibiotic production, intracellular protein cristals, adsorption of dyes). However, these changes are not always directly related with the potential of the bacteria to support the reproduction of the nematode. The nematode yields are often unpredictably low, also in cultures without any detectable presence of secondary form bacteria. It is therefore considered that characters changing with the phase shift are not necessarily related with a declining potential of the bacteria to support nematode yields. Objective of this study was to better understand the growth and metabolism of *P. luminescens* in continuous culture in a chemostat in order to improve the liquid culture of the nematode-bacterium complex.

Two parameters are used to control the growth in a chemostat -the dilution rate and the concentration of a limiting substrate. At low concentrations of an essential substrate the growth rate is proportional to the substrate concentration. However, at these low substrate concentrations the substrate is quickly used up by the microorganisms. In batch culture the exponential growth ceases at the moment

one essential nutritional elements is used up. In the chemostat the continuous addition of fresh medium containing the limiting substrate permits a continuous exponential growth. At the point where the microorgansism density in the chemostat reached a steady state the dilution rate equals the growth rate . This condition is not achieved at both, very high and very low dilution rates. At high dilution rates, the organisms cannot grow fast enough to keep up with the dilution and cells are washed out. At the other extreme, at very low dilution rates, a large fraction of cells may die from starvation, since the limiting nutrient is not added fast enough to permit maintenance of cell metabolism and the population will slowly be washed out.
The cell density in the chemostat is controlled by the concentration of the limiting substrate. If the concentration of the substrate is raised at a constant dilution rate, the cell density will increase although the growth rate will remain the same. Thus, by adjusting the dilution rate and the substrate concentration different population densities can be obtained at variable growth rates.
In the first experiment presented in this study the bacterial growth under various dilution rates and batch culture conditions were investigated in a glucose limited medium. The optical density, dry weight, bioluminescence activity and antibiotic production were determined. At the moment the culture reached a steady state one sample was tested with phase specific antibodies (Gerritsen *et al.*, 1995) to recognize phase variants. Objective of a second chemostat experiment was to induce the secondary form by changing the osmolarity of the medium (Krasomil-Osterfeld, 1995).

MATERIALS AND METHODS

The strain *Photorhabdus luminescens* PSH1 was isolated from dauer juveniles of *Heterorhabditis* sp. (HSH1). The bioluminescence activity (BLA) in $RLUxOD^{-1}s^{-1}$ (RLU = relative light units) was quantified with a luminometer (LUMAT LB 9501/16 Berthold). The antibiotic activity was assessed according to Drews (1982). The culture purity, stability and phase variation was monitored by frequent examinations for characteristic cell size, presence of inclusion bodies, colony morphology, pigmentation and dye uptake on Lab-Lemco and NBTA-Agar. Phase variation was further investigated by using phase specific antibodies. The phase variants were quantified by using cross-absorbed antisera (Gerritsen *et al.*, 1995) in indirect immunofluorescence cell staining tests as described by van Vuurde et al. (1983). The cell density was monitored spectrophotometrically at an OD of 725nm and by the dry weight to determine the steady-state.

Bacteria were grown in a medium, designed for growth under glucose-limiting conditions (Evans *et al.*, 1979). The glucose was autoclaved separately and added to the medium at room temperature together with a filter-sterilzed vitamin solution. The second chemostat experiment was started with YS-broth (Dye, 1968) reduced in the yeast extract concentration (2.5g/l; 190mOsmol) and latter Y-broth (without NaCl and 5g/l yeast extract; 50 mOsmol) was used. 0.1% antifoaming reagent silicone was added to the media.

A 1,5 l bioreactor (Meredos) connected to a bioreactor control system was used.

The inoculum was incubated overnight and then 45 ml were transferred into the chemostat. Before the continuous addition of the glucose or YS medium the cultures were incubated for 38 h and 17 h, respectively. The culture temperature was maintained at 25°C, the pH at 7.0. The pO_2-set point was 40% O_2-saturation at atmospheric pressure and culture temperature. The feeding was carried out by a membrane pump and the pumping off rate was controlled by a level control probe.

RESULTS AND DISCUSSION

Data obtained for the OD and the dry weight were always well correlated, the reason why the latter is not shown. The continuous culture 1 (Fig. 1) was started at a dilution rate of 0.06 h^{-1}. Theoretically, a chemostat reaches the steady state after 3 residence times (the residence time (T_r) is the reciprocal value of the dilution rate D, i.e., the T_r = 1/D for 6% dilution is theoretically 50 h). In the described experiment it took about 150 hours before the steady state was reached (Fig. 1). The variability of the OD values were probably due to a technical problems (failure of the stirrer, the pH probe and the level control) and consequently the bioluminescence varied accordingly. An increase of the dilution rate to 0.08 h^{-1} (generation time: 8.7 h), it took 50 h to reach steady state which coincides with the theoretically predicted duration. The dilution rate of 0.12 h^{-1} (generation time, 5.8 h) was run for 28 h and then increased to 0.16 h^{-1} (generation time, 4.3 h) for 63 h. A steady state at 12% was not awaited. The increase of the dilution rate resulted in a stepwise decrease of the OD and dry weight. Although the biomass reached a steady state at every tested dilution rate, a constantly high biomass could not be maintained. After 63 h at 16% dilution rate the media supply was terminated. These batch culture condition resulted in an increase of the biomass. When the medium supply was taken up again the OD and dry weight decreased like documented before.
Comparing the bioluminescence activity during the steady state at 6, 8 and 16% dilution rate (12% conditions too short to reach steady state) it decreased with increasing dilution rates. Batch culture condition caused an almost total termination of the bioluminescence activity which could be reestablished by starting the media supply again. If the bioluminescence is evaluated independendly from the steady state an extreme oscillation is obvious. Similar results were recorded in batch cultures. The bioluminescence activity increases until 15 h after inoculation and the maximum activity coincides with the end of the exponential growth phase. No constant values were recorded after the period of maximum activity (data not shown). The bioluminescence activity is always highly variable. Thus, a quantification of phase variants by measuring the bioluminescence activity is not recommended. However, it is a good indicator for the growth activity and condition of the culture. Technical failures and or detrimental growth conditions are first indicated by decreasing light production.

The production of antibiotic substances was recorded qualitatively. It started after setting up the dilution rate to 6% and was continuously recorded until 53 h and 150 h after the medium supply had been stopped.

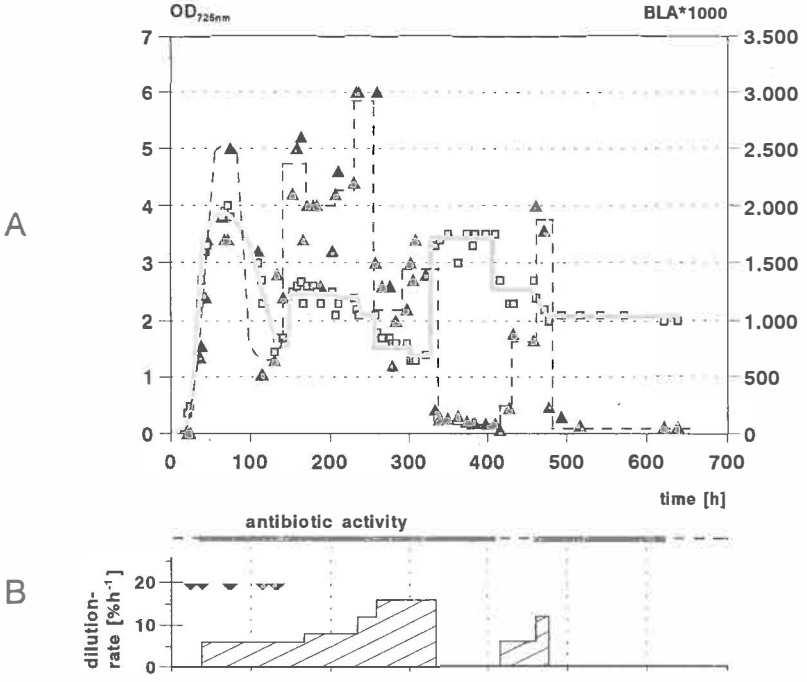


Fig. 1: Continuous culture of *Photorhabdus luminescens* in a glucose limited medium at variable dilution rates and batch culture conditions over a period of 638 h at 25°C, 40% pO_2 and pH 7. A.: OD at 725 nm (squares and full line) and bioluminescence activity BLA (triangles and dotted line). For better presentation lines were drawn with significant tendencies. B.: Antibiotic activity (+: bold line; -: dotted line), dilution rate (bars) and time at which technical problems occured (triangles). Primary form, but not secondary form specific antibodies labelled cell samples (n = 7) taken at the different steady states reached after adjustment of variable dilution rates or batch conditions.

The indirect immunofluorescence cell staining test with the form specific antibodies revealed the presence of primary form cells throughout the whole experiment.

The growth in a complex medium (YS and Y broth) was tested in a second continuous culture (Fig. 2). The cell density in the complex media was lower compared to the density in experiment 1, probably due to the lack of sodium-chloride in the Y medium. The change of the osmolarity (at 99 h) did not induce the loss of the antibiotic and bioluminescence activity recorded at 85 h after inoculation. However, it may be speculated that the period of non-production had been prolonged by the changing osmolarity. Like in the first experiment the bioluminescence and antibiotic activity is lost under batch culture conditions. By feeding with Y-broth the OD and dry weight doubled due to the higher yeast extract content.

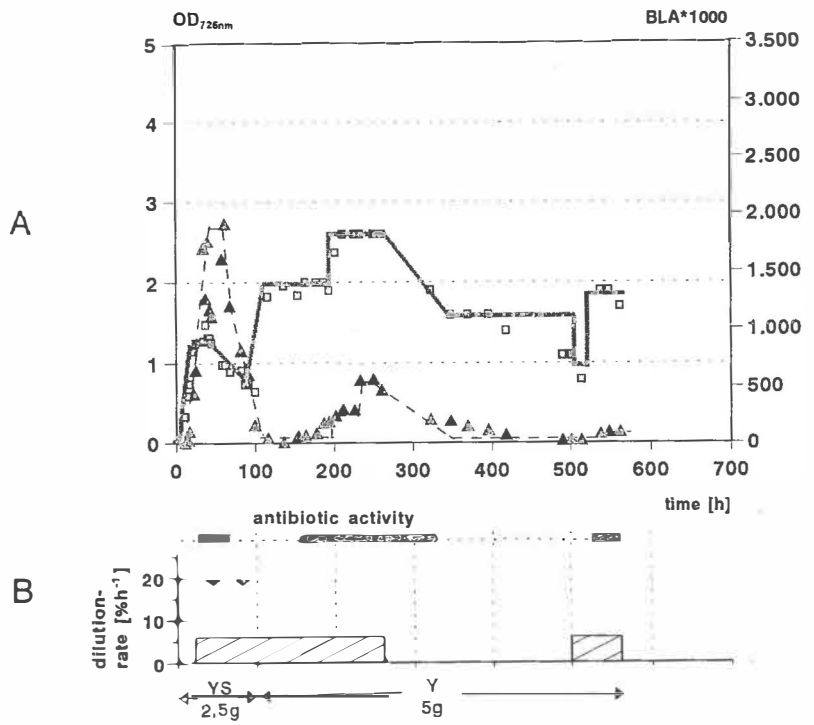


Fig. 2: Continuous culture of *Photorhabdus luminescens* in YS (2.5g/l yeast extract; 190 mOsmol) and Y broth (without NaCl and 5g/l yeast extract; 50 mOsmol) at 6% dilution rates and batch culture conditions over a period of 562 h at 25°C, 40% pO_2 and pH 7. A.: OD at 725 nm (squares and full line) and bioluminescence activity BLA (triangles and dotted line). For better presentation lines were drawn with significant tendencies. B.: Antibiotic activity (+: bold line; -: dotted line), dilution rate (bars) and time at which technical problems occured (triangles). Primary form, but not secondary form specific antibodies labelled cell samples (n = 15) taken at the different steady states reached after adjustment of 6% dilution rate or batch condition.

Neither the decreasing osmolarity nor the changing growth conditions from a dilution rate of 0.06 h^{-1} to batch culture condition induced a phase shift to secondary form according to the results obtained with the antibodies. 11 samples could be labelled with the primary form but not with the secondary form specific antibody. 4 samples could not be labelled with either of the two antibodies. The cultures used to produce the specific antibodies were highly selected forms. Their physiology is therefore not necessarily corresponding with the physiological status of variants growing in batch or continuous cultures. The results also indicate that cells other than those labelled by the available antibodies exist.

It can be concluded that the loss of typical primary form characters like bioluminescence activity and antibiotic production are not correlated with the occurance of cells which can be labelled with secondary form specific antibodies. Whether the continuous growth conditions of *Photorhabdus luminescens* can support nematode reproduction and development remains to be proven by future experiments. Modelling of growth and metabolism in the chemostat should provide fundamental information for an overall improvement of the nematode-bacterium liquid culture.

REFERENCES

Dye, D.W. (1968). A taxonomic study of the genus *Erwinia*: I. The "amylovora" group. N.Z.J. Sci. **11**, 590-607.

Drews, G. (1983). Quantitative Bestimmung der Antibiotica. In: Mikrobiologisches Praktikum. 4. Aufl. Springer-Verlag Berlin Heidelberg, 168-177.

Evans, C.G.T., Herbert, D., and Tempest, D.W. (1979). The continuous cultivation of microorganisms. Construction of a chemostat. In: Methods of Microbiology, Vol.2, 278-324.

Gerritsen, L.J.M., van der Wolf, J.M., van Vuurde, J.W.L., Ehlers, R.-U., Krasomil-Osterfeld, K.C., and Smits,P.H. (1995). Polyclonal antisera to distinguish strains and form variants of *Photorhabdus* (*Xenorhabdus*) *luminescens*. Appl. Environm. Microbiol. **61**, 284-289.

Krasomil-Osterfeld, K. (1995). Induction of phase shift of *Photorhabdus luminescens,* strain PSH1 by low osmolarity. Proc. Cost 819 Workshop, Debrecen, Hungary, 27 May - 2 June, 1994.

van Vurde, J.W.L., van den Bovenkamp, G.W., and Birnbaum, Y. (1983). Immunofluorescence microscopy and enzyme-linked immunosorbent assay as potential routine tests for the detection of *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas campestris* pv. *phaseoli* in bean seed. Seed Sci.Technol. 11, 547-559.

HOST FINDING BY THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA FELTIAE*

A. PETERS[1], K. HUNEKE[2] & R.-U. EHLERS[2]
[1] Ecogen Europe, Parco Tecnologico Agro-Alimentare dell' Umbria, 06050 Todi (PG), Italy
[2] Dept. Biotechn. and Biol. Control, University Kiel, 24223 Raisdorf, Germany

Summary

The ability of *Steinernema feltiae* to disperse in sand and to locate *Tipula oleracea* L4 and *Galleria mellonella* last instar larvae was tested in a series of experiments. Destructive sampling of sand columns showed, that 50% (approx.) of dauer larvae (DL) dispersed equally without the presence of an insect host. The presence of *T. oleracea* larvae >= 4cm from the nematode inoculation site did not increase the proportion of DL dispersing. However, the nematodes did accumulate in the vicinity and inside the *T. oleracea* larvae. When DL were allowed to choose between two sand filled cylinders, one with one without an insect inside, the number of nematodes, which incidentally visited the cylinder without an insect was not significantly lower than the number of nematodes aggregating in the cylinder with the insect. Hence, *S. feltiae* DL were obviously not attracted by the insect but were arrested near the insect after having incidentally reached it. There was no difference in the aggregation of DL in response to *T. oleracea* or *G. mellonella* larva, but DL penetration into *G. mellonella* was significantly higher than penetration into *T. oleracea*. Hence, the host specificity of *S. feltiae* DL seems to be related to their penetration activity rather than their host finding ability.

Introduction

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are effective biological control agents against a variety of soil living pest insects (Klein, 1990). Their ability to locate the pest insect is often emphasised as one of their major advantages. Although nematodes have been shown to be attracted by a large variety of insect released cues (Ishibashi & Kondo, 1990), the mechanisms of insect host location in the soil are still poorly understood. It is widely assumed that the nematodes are attracted by gradients of insect released cues, whereas incidental dispersal and arrestment near the host has seldomly been considered. Both strategies, attraction and arrestment, would result in nematode aggregation around the insect hosts. To examine the dispersal and the host finding ability of *Steinernema feltiae* in sand, a series of experiments were conducted. One of the experiments was particularly designed to differentiate between nematode attraction and arrestment.

Materials and Methods

Dauer larvae (DL) of *Steinernema feltiae* strain OBSIII were reared in *G. mellonella* larvae (Dutky *et al.*, 1964) and stored in tap water at 6°C. Unless otherwise stated they were used for experiments within 2 days of being harvested. Nematode dispersal was assessed in 10cm long columns filled with sterilised silica-sand (grain size: 200-400μm, 15% moisture w/w). The DL distribution within the sand columns was assessed by extracting the nematodes from 5 x 2cm wide sections of the columns. Sections were designated with numbers 0 to 4 with 0 being the section where DL were applied to and 1 to 4 the adjacent sections with increasing distance. Nematodes were extracted by suspending each section in a 10-fold volume of tap water and then decanting the water and nematodes after letting the sand settle for 30 seconds. This procedure was repeated once. The nematodes were then concentrated on a 15 μm sieve and counted.
In **experiment 1**, the columns were 2.5cm in diameter (dm) and insect hosts were not present in the sand columns. Ten columns were prepared and 500 DL in 0.5ml water were applied to one end of each column (section 0). The columns were stored horizontally at room temperature (20-23°C). The experiment was done with 1 day old and with 5 months old DL. After periods of 2, 4, 6, 8 and 24 hours, two columns were sampled and nematodes were extracted from each 2cm section and counted.

In **experiment 2**, DL migration was assessed in the presence of *Tipula oleracea* L4 larvae using 4cm wide sand columns. The larvae were fixed at 3 different distances from section 0 where 2000 DL in 1ml tap water were applied. Columns without insects were prepared as a control. For each variable 3 columns were prepared. They were preincubated for 1 hour before adding the nematodes. After 24 hours incubation in horizontal position at 20-23°C nematodes were extracted from each section and counted. In addition the nematodes that had penetrated into the insect were counted by dissecting the larvae three days later.
Experiment 3 was done to determine whether DL are attracted to hosts or reach their hosts incidentally. Petri dishes (8.5cm dm) were filled with a 5mm layer of moist silica sand (200-400μm grain size, 15% moisture w/w) and two plastic cylinders (1cm dm) were placed vertically on each Petri dish at 4cm apart. The cylinders (3cm long) were closed by a 200μm Nylon mesh at the bottom and also filled with moist sand to 1cm height. One of the cylinders contained either a *T. oleracea* L4 larva (1st variable), a *G. mellonella* last instar larva (2nd variable), or no insect (control) whereas the opposite cylinder for each variable was only filled with sand. After a preincubation period of 1 hour, 500 DL in 0.5ml tap water were put in the centre of each dish equidistant from the two cylinders. The Petri dishes with cylinders were incubated for 20 hours at 20°C. The cylinder without the insect was emptied every hour up to 20 hours and DL were extracted by washing the sand in water (see above). It was then refilled with sand and put back in the same position. The opposite cylinder was also lifted every hour to allow for equivalent conditions. The nematodes in this opposite cylinder were extracted only once at the end of the 20 hour period. The insects were dissected after 3 days reincubation at 20°C to count the nematodes that had entered them. The cumulative counts of DL in the cylinder without the larva was regarded as an estimate for the number of nematodes, which have visited this cylinder over the 20 hours period.

Results

Experiment 1
The spontaneous dispersal of *S. feltiae*, without insect larvae, is shown in Fig. 1a,b. A large proportion of the DL remained in section 0 where they were applied. Of the 1 day old DL, after 4 hours, 50% (approx.)

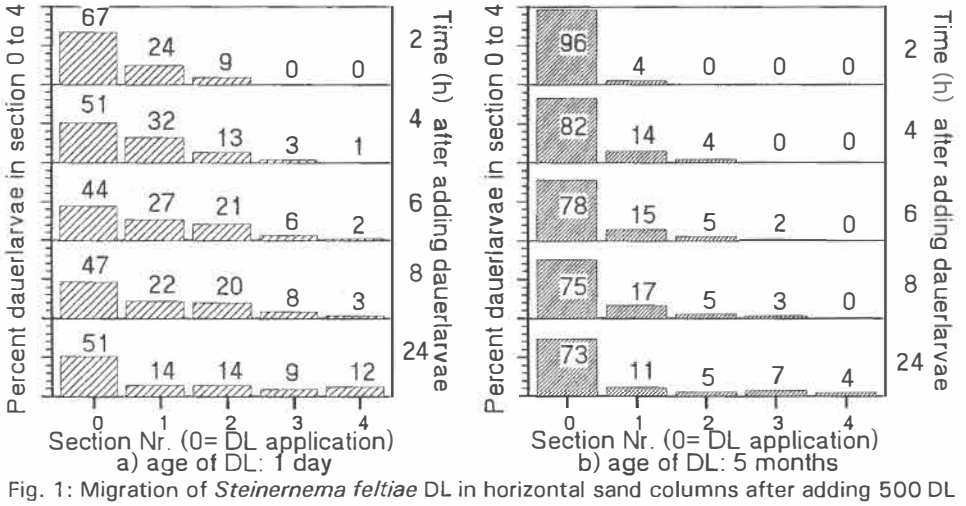


Fig. 1: Migration of *Steinernema feltiae* DL in horizontal sand columns after adding 500 DL on section 0 (n = 2, each time period).

had dispersed from section 0 and there was no further dispersal throughout the experiment (24 hours) (Fig. 1a). This implies that 50% of the DL would not disperse. The nematodes, which had dispersed were found equally distributed between sections 1-4 after 24 hours (Chi-square test, p=0.1, Fig. 1a,b). The proportion of non-dispersing DL was significantly larger for the 5 months old DL than for the 1 day old DL. The

active 5 month old DL, however, were also found equally distributed over the remaining sections after 24 hours (Chi-square test, p=0.1, Fig. 1b).

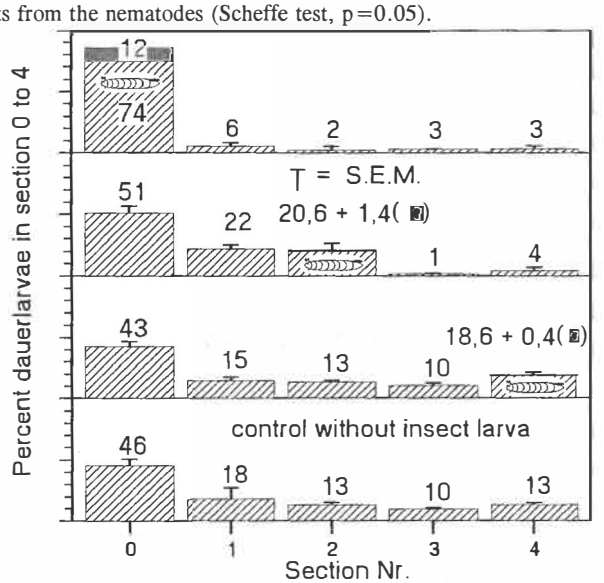
Experiment 2

In sand columns with larvae of *T. oleracea*, the proportion of nematodes dispersing from the application point (section 0) was not significantly larger than in the control (ANOVA, p=0.05, Fig. 2). However, when the larva was fixed in section 0, the proportion of nematodes leaving this section was significantly lower (ANOVA, p=0.05, Fig. 2, 1st row), indicating that the DL are arrested in the vicinity of a potential host. In columns with the host insect in section 2, i.e. 4cm distant from the DL application point, 49% of the DL dispersed and were mostly found in section 1 (22%) and 2 (22%) (Fig. 2, 2nd row). Only few nematodes (5%) migrated beyond the section with the insect. Thus, the distribution of nematodes in sections 1-4 was not equal (Chi-square test, p=0.05). This suggests that the DL passing the section with the insect are arrested around the insect. When the insect was fixed in section 4, the proportion of DL in this section also was increased slightly, however, nematode distribution in sections 1 to 4 was not significantly different from equal (Chi-square test, p=0.1). The percentage of nematodes penetrating the leatherjackets decreased significantly with the distance of the insects from the nematodes (Scheffe test, p=0.05).

Experiment 3

There was no significant difference in the cumulative number of DL extracted hourly from the insect free cylinder and the number of nematodes extracted after 20 hours from the cylinder with the insect larvae (Wilcoxon test, p = 0.05, Fig. 3). This was observed both host insects used, *T. oleracea* and *G. mellonella*. In the control, the cumulative number of hourly extracted DL was significantly higher than the number found in the opposite cylinder after the 20 hours incubation period (Wilcoxon test, p=0.05, Fig. 3). This suggests that the DL migrate into the cylinders incidentally and only stay, if there is a suitable insect host present.

It is worth noting that despite there being no significant difference in the number of *S. feltiae* accumulating in cylinders with *G. mellonella* or with *T. oleracea* L4 (Mann-Whitney test, p=0.1) nematode penetration into *G. mellonella* larvae was significantly higher than into *T. oleracea* (Mann Whitney test, p=0.01).



nematodes inside insect nematodes in sand

Fig. 2: Migration of *Steinernema feltiae* DL in horizontal sand columns with *Tipula oleracea* at different distance 24h after adding 2000 DL on section 0 (n=3, each variable).

Discussion

These experiments showed, that the *S. feltiae* population is composed of an actively dispersing and a non-dispersing part, and the proportion of non-dispersing DL increases with nematode age. Similarly, a division of nematode populations into behavioural distinct groups was noticed for the infectivity of several entomopathogenic nematode species (Fan & Hominick, 1991b), and fluctuations in the proportion of infective DL with age other than the decline attributed to the depletion of food reserves have been reported (Fan & Hominick, 1991a; Griffin, 1994). It can not yet be decided, whether such fluctuations also occur with nematode migration activity.

When nematode aggregation was assessed, a distinction was seldomly made between nematode attraction or arrestment (Ishibashi & Kondo, 1990). The data shown indicate that DL of *S. feltiae* find their insect hosts incidentally by undirected migration. If nematodes were attracted by an insect released gradient, the number in the cylinder with the insect (experiment 3) would have exceeded the cumulative number of nematodes found in the insect-free cylinder. Hence, *S. feltiae* DL only seem to recognise hosts in close vicinity and penetrate into the host or remain around their hosts. Judging from the lack of an increased dispersal in with an insect at 4cm distance the critical distance for *S. feltiae* to recognise a host would be less than 4cm (experiment 2).

If DL accumulate around the host after having incidentally reached it, the DL behaviour should change in response to insect cues. Lewis *et al.* (1992) showed, that *S. glaseri* does change its behaviour from long range to localised search after exposure to host cues but no behavioural change was observed in *S. carpocapsae*. The results of these experiments indicate that the dispersing proportion of an *S. feltiae* population might react in a similar way to *S. glaseri* and hence falls in the category of 'cruise foraging' (Lewis *et al.*, 1992) and the non-migrating part appears to adopt the 'ambush foraging' strategy (*sic*). We thus find cruise *and* ambush foragers in the same *S. feltiae* population and the proportions of each foraging type changes with nematode age.

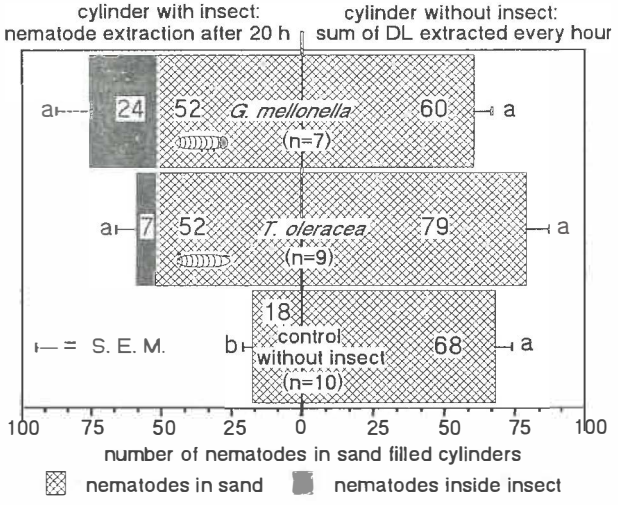


Fig. 3: Migration of *Steinernema feltiae* DL into opposite sand filled cylinders after adding 500 DL in the middle between them.

Aggregation of *S. feltiae* seems to be rather unspecific, since the nematodes do equally accumulate at *G. mellonella* and *T. oleracea* larvae although *T. oleracea* is much less susceptible to *S. feltiae* (Peters & Ehlers, 1994). The pronounced differences in nematode penetration into the two insect species indicate, that host specificity in *S. feltiae* is mainly due to differences in their penetration activity.

In the soil environment unspecific volatile host cues, like CO_2, are released by many sources other than insect hosts like plant roots or lumps of decaying organic matter. Hence, long range attraction of DL to CO_2 sources would not necessarily result in host contact in the soil. Therefore, undirected dispersal is not necessarily a less efficient host finding strategy than directed migration towards unspecific insect volatiles.

References

DUTKY, S. R., THOMPSON, J. V. & CANTWELL, G. E. 1964. J. Insect Pathol. **6**: 417-422.
FAN, X. & HOMINICK, W. M. 1991a. Revue Nématol. **14**: 407-412.
FAN, X. & HOMINICK, W. M. 1991b. Revue Nématol. **14**: 381-387.
GRIFFIN, C. T. 1994. VIth Int. Coll. Invertebr. Pathol. and Micr. Control, Montpellier, France, p. 14.
ISHIBASHI, N. & KONDO, E. 1990. In: Gaugler, R. & Kaya, H. K. (Eds.): *Entomopathogenic nematodes in biological control*, CRC Press, Boca Raton, USA, 130-150.
KLEIN, M. 1990. In: *as previous*, pp. 195-214.
LEWIS, E. E., GAUGLER, R. & HARRISON, R. 1992. Parasitology, **105**: 309-315.
PETERS, A. & EHLERS, R.-U. 1994. J. Invertebr. Pathol. **63**: 163-171.

STORAGE OF ENTOMOPATHOGENIC NEMATODES OF THE GENUS *HETERORHABDITIS* AT TWO TEMPERATURES. EFFECT ON INFECTIVITY, ENERGY RESERVE AND NUMBER OF BACTERIA

KERSTIN JUNG

Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany

Introduction

Entomopathogenic nematodes have been successfully introduced to many markets. However, the unsatisfactory shelf-life, in particular of heterorhabditid nematodes, is still a major obstacle for their commercialisation (e.g. Richards & Rodgers, 1990). Unlike other biopesticides, for example toxin-based *Bacillus thuringiensis*-products, the infective juveniles of *Heterorhabditis* age with time. This is expressed in a depletion of energy reserve and a loss in viability. Besides the conditions during production that determine the initial state of nematodes, many abiotic and biotic factors (e.g. temperature and contaminations) can affect shelf-life and fitness of the infective juveniles during storage. Both the aging process and the influence of different factors on properties of the nematodes, responsible for their quality, are poorly understood. Additionally, the role of the bacteria associated with the nematodes within the aging process is unknown.
The objective of this research was to study both energy reserve of the infective juveniles and the fate of symbiotic bacteria in comparison to infectivity during storage at two temperatures.

Material and methods

NEMATODES

The isolates GB-H-Uk211 (NW European group) and IRL-H-K122 (Irish group) of *Heterorhabditis* sp., *H. bacteriophora* HI82 and *H. zealandica* NZH3 were propagated on larvae of the greater wax moth, *Galleria mellonella*, according to standard procedures (e.g. Poinar, 1975). First tests were performed without storing the nematodes. Thereafter, one part of each batch was stored in non-sterile tap water in a tissue culture flask at 5±2 °C, whereas another part was stored at 20±2 °C.
The bioassay on infectivity and the sampling for the measurements of the energy reserve were both carried out at weekly intervals. The number of bacteria was assessed every two weeks.

INFECTIVITY

Infectivity was assessed in plastic containers (9 cm height, 3 cm Ø) filled with moist sand [8 % (v/w)] and a *Galleria* larva at the bottom. 100 infective juveniles were inoculated on top of each container and incubated at 15 °C for eight hours. The insects were then removed from the sand, washed and incubated at 20 °C. After four days mortality was recorded.

ENERGY RESERVE

As a measure of the energy reserve, both triglyceride and glycogen content of the nematodes were assessed in a spectrophotometrical way.

To determine the triglyceride content, the nematodes were manually homogenised using a microhomogenizer (Hearse, . 1984). The lipids were extracted with chloroform and methanol. The triglyceride content was measured after incubation with an enzymatic test solution.
Glycogen content was determined with the Anthron reagent (Seifter et al., 1950). The infective juveniles were digested with potassium hydroxide and glycogen was precipitated with ethanol. After incubation with the Anthron reagent, the glycogen content was measured.

NUMBER OF BACTERIA

The number of bacteria was assessed as colony forming units (CFU). Twenty living infective juveniles were individually crushed on glass slides. The homogenate was diluted and each dilution was plated onto three nutrient agar plates using a spiral plater. The plates were incubated at 24 °C and the number of CFU was assessed after 72 hours.

Results and discussion

INFECTIVITY

The results of the infectivity assay are shown in **Table 1**. In the first week, infectivity of all isolates was low. During the following weeks infectivity increased, except for NZH3 stored at 5 °C. Generally, the infective juveniles stored at 20 °C reached higher levels of infectivity than those stored at 5 °C. Furthermore, HUk and HK122, stored at 20 °C, reached the maximum of infectivity earlier than when stored at 5 °C.

Table 1. Percentage infectivity of *Heterorhabditis* sp. HUk and HK122, *H. zealandica* NZH3 and *H. bacteriophora* HI82 right after emergence from an insect host (week 1) and after storage at either 5±2 °C and 20±2 °C at 15 °C. (n.t. = not tested)

week	HUk		HK122		NZH3		HI82	
1	25		20		0		0	
	5 °C	20 °C	5 °C	20 °C	5 °C	20°C	5 °C	20 °C
2	70	80	30	70	0	0	0	0
3	60	90	25	**100**	0	**25**	**20**	0
4	75	**100**	**40**	70	0	25	10	**30**
5	70	90	30	75	0	25	0	25
6	**90**	100	20	75	n.t.	25	0	25
7	80	90	30	90	n.t.	0	0	10

HUk was the only isolate that could cope with the rough test conditions (15 °C, 9 cm to migrate within eight hours) regardless of the storage temperature. Isolates of the NW European group have been proven to be excellent migrators (Westerman, 1995). They are also more adapted to cold climates than isolates of *H. zealandica* and *H. bacteriophora* that derive from warmer regions. Nevertheless, considering that HK122 should also be adapted to cold temperatures, it seems that the low storage temperature suppresses the pathogenic action of infective juveniles. In *Steinernema,* Fan and Hominick (1991) reported an initial decline in infectivity for *Galleria* larvae at 15 °C when the infective juveniles were stored at 5 °C. However, after six weeks, infectivity started to increase again whereby, the initial level was reached after 16 weeks. The same authors

concluded that low temperatures induce a state in which the infective juveniles lose their ability to parasitise a host.
Calculations based on the initial nematode concentration revealed that after seven weeks of cold storage, high percentages of infective juveniles of all isolates, except HUk, died (HUk 16 %, HI82 33 %, HK122 40 % and NZH3 69 %). However, mortality of the infective juveniles cannot be the only explanation for the level of infectivity. Although 70 % of the infective juveniles of HK122 stored at 20 °C died, the ones that were alive could infect 90 % of the test insects.

ENERGY RESERVE

After seven weeks of storage at 5 °C, the triglyceride content of HI82 and HK122 was still as high as the initial level of 34 µg. Whereas, HUk and NZH3 had used about 40 % of their neutral fat reserve (triglyceride content of HUk and NZH3 declined from 58 to 33 µg and from 44 to 29 µg, respectively).
At 20 °C storage temperature, HK122 again could preserve its triglyceride content; only 15 % of the initial amount was used during seven weeks, whereas HI82 and NZH3 took access of 40 % and HUk of 50 % of their initial triglyceride content.
Glycogen content of HUk, HK122 and NZH3 increased at both storage temperatures. The extent of this increase was the same at both storage temperatures for HK122 (6 µg initially and 9 µg after seven weeks), but greater at 20 °C than at 5 °C for HUk and NZH3 (6 and 3 µg initially and after seven weeks 15 and 11 µg at 20 °C, compared to 11 and 9 µg at 5 °C, respectively). Glycogen content of HI82 was identical to the initial amount of 3 µg after seven weeks at both storage temperatures.
In the present study cold storage did not show the preserving effect on the energy reserve and viability of HUk, HK122 and HI82 known from previous experiments (unpubl. data).
On the one hand, if these physiological assessments are taken into account, the remaining high triglyceride content of HK122 after seven weeks at 20 °C, could have contributed to its high level of infectivity, although a lot of the specimens died. On the other hand, the decline of the triglyceride content of HUk at both storage temperatures, did not show up in infectivity. Probably the high glycogen content of HUk could have replaced the triglycerides as a main energy reserve. The increase of the glycogen content, accompanied by a decrease of the triglyceride content, suggests that the infective juveniles resynthesize glycogen from triglycerides.
The increase of the glycogen content, as well as the increase in infectivity, give reason to believe that temperature-dependent development processes occur in heterorhabditids like in other parasitic nematodes (Bolla, 1980).

NUMBER OF BACTERIA

The results of the assessments of bacteria per infective juvenile are summarized in **Table 2**. There was a high heterogeneity between the specimens of one treatment, between treatments and between the nematode isolates. Generally, the highest numbers of CFU (up to 2600) were obtained with HUk and HK122 stored at 20 °C. In contrast, higher numbers of CFU per infective juvenile of NZH3 were found when stored at 5 °C, compared to 20 °C. HI82 did not exhibit high numbers of CFU at neither storage temperature. Except for HK122 stored at 20 °C at two assessment days, there were always some specimens apparently free of bacteria. In the case of HI82 this even was the majority.
The presented results confirm previous observations of a temperature-dependent increase in number of bacteria per infective juvenile (Jung, 1994). Obviously, the number of bacteria did not directly influence the infectivity, i. e. comparable numbers of bacteria

did not result in comparable levels of infectivity; after seven weeks, HK122 and HUk stored at 5 °C contained a mean of about 300 bacteria per infective juvenile, but infectivity was 30 and 80 %, respectively. However, if the bacteria multiply inside the intestine of the nematode, they could have an effect on the energy reserve of the infective juvenile. Future research will focuse on the relationship between the two symbiotic partners.

Table 2. Number of bacteria per infective juvenile (mean of 20 specimens and range in parenthesis) of *Heterorhabditis* sp. HUk and HK122, *H. zealandica* NZH3 and *H. bacteriophora* HI82, measured as colony forming units, right after emergence from an insect host (week 1) and after storage at either 5±2 and 20±2 °C.

week	HUk		HK122		NZH3		HI82	
1	291 (0-708)		257 (0-606)		188 (0-1792)		0,8 (0-5)	
	5 °C	20 °C	5 °C	20 °C	5 °C	20°C	5 °C	20 °C
3	270 (0-704)	441 (0-1870)	305 (0-574)	531 (0-1268)	379 (0-1842)	224 (0-731)	1 (0-5)	2,5 (0-5)
5	365 (0-861)	414 (0-1241)	253 (0-667)	742 (28-2055)	173 (0-454)	36 (0-370)	0,3 (0-5)	0,3 (0-5)
7	330 (0-898)	797 (0-2620)	330 (28-592)	763 (0-2639)	131 (0-667)	81 (0-398)	0,3 (0-5)	0,8 (0-5)

Acknowledgements

This study was financed by the German Research Organisation (DFG). I would like to thank Dr. H. Bathon for his support and Ms R. Schümann and Ms N. Fischer for technical assistance.

References

BOLLA, R., (1980). Nematode energy metabolism. In: Zuckerman, B.M. (Ed.). Nematodes as Biological Models. New York, Academic Press : 165-192.

FAN, X. & HOMINICK, W.M., (1990). Effects of low storage temperature on survival and infectivity of two *Steinernema* species (Nematoda: Steinernematidae). Revue Nématol., **14**: 407-412.

HEARSE, D.J., (1984). Microbiopsy metabolite and paired flow analysis: a new rapid procedure for homogenisation, extraction and analysis of high energy phosphates and other intermediates without any errors from tissue loss. Cardiovasc. Res., **18**: 384-390.

JUNG, K., (1994). The symbiotic relationship between *Heterorhabditis* sp. and its bacterium, *Photorhabdus luminescens*, influenced by storage at two different temperatures. Abstracts, 6th Int. Colloq. Invert. Pathol., Montpellier, France, 278.

POINAR, G.O., (1975). Entomogenous nematodes. Leiden, Brill, 317 p.

RICHARDS, M.G. & P.B. RODGERS, (1990). Commercial development of insect biocontrol agents. Aspects of Applied Biology, **24**: 245-253

SEIFTER, S., DAYTON, S., NOVIC, B. & MUNTWYLER, E., (1950). The estimation of glycogen with the Anthron reagent. Arch. Biochem., **25**: 191-200.

WESTERMAN, P.R. (1995). Comparative vertical migration of twenty one isolates of the insect parasitic nematode Heterorhabditis spp. in sand at 20 °C. Fundam. appl. Nematol., **18**: 149-158.

Preservation of natural beneficial traits under laboratory conditions: The case of IS5, a heat tolerant isolate of *Heterorhabditis bacteriophora*

David I. Shapiro[1], Itamar Glazer[1], and Daniel Segal[2]

[1] Department of Nematology, Institute of Plant Protection, Volcani Center, Bet Dagan 50250, Israel.

[2] Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Tel Aviv 69978, Israel.

ABSTRACT

Natural populations of entomopathogenic nematodes may possess novel traits that offer advantages in biological control. If, however, the populations cannot be reared under laboratory conditions, and if their beneficial traits are unstable, then increases in biocontrol efficacy will be minimal. The stability of heat tolerance in, and fitness under laboratory conditions of, a newly discovered strain of *Heterorhabditis bacteriophora* (designated "IS5") were investigated. Trait stability and fitness assays were conducted after multiple passages through *Galleria mellonella*. Assays were conducted on IS5 populations reared at 30°C and 25°C. Trait stability was determined as survival at elevated temperatures. Relative to the commercial strain of *H. bacteriophora* (HP88), the IS5 strain exhibited greater heat tolerance after 12 passages regardless of selection pressure. The population of IS5 reared at 30°C exhibited greater heat tolerance than the population reared at 25°C. Results indicated that the heat tolerance trait is genetically based. The virulence, infectivity, and reproductive potential of IS5 nematodes were greater than or equal to that of HP88 nematodes. The heat tolerance trait in IS5 was retained after 6 weeks of storage.

INTRODUCTION

Sensitivity of entomopathogenic nematodes to extremes of the environment constitutes a major impediment to exploitation of their full biocontrol potential, and to expansion of their application to new habitats. In particular, the intolerance of steinrnematids and heterorhabditids to heat, desiccation and solar radiation reduces their viability and leads to erratic results in the field (Kaya and Gaugler, 1993). Increasing their tolerance to harsh environments will enhance the efficacy of these nematodes. In addition it will allow to expand their application to exposed surfaces, such as foliage, which are inhabited and damaged by many insect pests. Genetic improvement has been a successful approach for generating crops and livestock with desired traits. During the past several years we have started to explore this approach for generating strains of entomopathogenic nematodes with enhanced tolerance to extreme environmental conditions. Genetic improvement can be attempted through several avenues. Selection for lines displaying enhanced tolerance, e.g. to heat, can be successful if appreciable genetic heterogeneity underlying heat tolerance exists in the original population (Glazer et al., 1991). Alternatively, mutagenesis can be employed and individuals displaying enhanced tolerance can be screened among the progeny of individuals treated with a mutagene. We have demonstrated the feasibility of this

approach for generating morphological mutants in *Heterorhabditis bacteriophora* (Zioni et al., 1992; Koltai et al., 1994). Still another possibility is to assemble a collection of isolates of nematodes from a variety of habitats, particularly those with severe environmental conditions, and assay them for enhanced tolerance. This latter approach is exemplified in the present paper.

These avenues of genetic improvement may fail if the trait being selected or screened for is unstable under laboratory rearing conditions (Gaugler, 1987). Moreover, selection for strains displaying one enhanced trait can result in reduction in overall efficacy (Hopper et al., 1993). Here we describe a new heat tolerant strain of *H. bacteriophora*, and demonstrate the preservation of the heat tolerance trait under laboratory rearing conditions. Further, we find that the apparent efficacy of this strain, as determined by examination of various parameters of general fitness (e.g. virulence, infectivity, reproductive capacity, and survival in storage), has not been compromised. A full account of our results will be published elsewhere (Shapiro et al., 1995).

RESULTS

During surveys in the Negev desert in Israel a heat tolerant strain designated IS5 was discovered. Based on morphological criteria we tentatively assigned it to the *Heterorhabditis bacteriophora* Poinar species. Relative to the commercial strain of *H. bacteriophora*, HP88, the survival rate of the IS5 strain was more than five fold greater when exposed to elevated temperatures (36°C) for 8 hours.

Trait preservation: Our first objective was to examine whether the heat tolerance trait in IS5 is preserved under laboratory rearing conditions. Following multiple passages through the last instar of *Galleria mellonella* (Woodring and Kaya, 1988) with or without selection pressure i.e. at 30°C or 25°C, respectively, trait stability was evaluated by measuring nematode survival after exposure to 40°C for 2 h. The HP88 commercial strain (also reared at 30°C or 25°C) served as control. The HP88 strain was unable to reproduce at 30°C beyond four passages, and was therefore excluded from the analyses reported herein. For convenience, the three nematode populations that were tested will be referred to as I_{30}, I_{25} and H_{25} representing the IS5 strains reared at 30°C and 25°C, and the HP88 strain reared at 25°C, respectively.

Nematode survival at 40°C after 12 passages through *G. mellonella* larvae was greater in the IS5 strain than the HP88 strain regardless of selection pressure. For H_{25}, percent survival was 3.3±1.7, as compared to 56.1±4.6 and 95.6±1.1 for the I_{25} and I_{30} populations, respectively. Comparable results were obtained already after 8 or 10 passages. Much of the survival ability of I_{25} nematodes was recovered after they were transferred to 30°C for six additional passages, and recently we have shown that the I_{30} population has retained its heat tolerance trait after 20 passages (data not shown).

Preservation of general fitness: Our next objective was to examine overall fitness of the heat tolerant IS5 strain. The following parameters of fitness were studied: virulence, infectivity, reproductive potential, and storage capacity. Fitness was measured

in I_{25} and I_{30} after 12 passages through *G. mellonella*, and in H_{25} nematodes.

Virulence was determined after 12 passages at 25°C or 30°C, by measuring mortality of *G. mellonella* larvae 6-48 h after exposure to infective juveniles at either 25°C or 30°C. Both IS5 and HP88 caused larval mortality at a faster rate in 30°C than at 25°C (data not shown). After 30 h of exposure at 30°C the I30 nematodes caused significantly greater mortality of *G. mellonella* than the H25 population (78.5% vs. 23.8% respectively). This difference was lost however at 48 h of exposure.

Infectivity was examined by counting the number of nematodes that infected *G. mellonella* larvae using the pepsin digestion method (Mauleon et al., 1993). Infectivity of IS5 was not different from the control HP88 nematodes within either of the temperatures tested. Interestingly, a greater number of nematodes from the I_{30} population infected *G. mellonella* larvae at 30°C than at 25°C (average of 27.1±7.1 and 1.9±0.4 nematodes/larva, respectively).

Reproductive potential was assessed by monitoring the number of infective juvenile progeny resulting from cadavers of *G. mellonella* (originating from the virulence assays) maintained at 25°C or 30°C. Nematodes from IS5 reproduced at higher rates at 30°C than at 25°C (mean respective number of progeny produced per larva was $132\pm10x10^3$ and $67\pm27x10^3$ respectively for I_{30}; and $106\pm6x10^3$ and $77\pm13x10^3$ respectively for I_{25}). Reproduction of the control strain HP88 reared at 25°C was less affected by temperature ($84\pm10x10^3$ progeny at 30°C and $51\pm10x10^3$ progeny at 25°C).

Storage capacity was determined according to the procedure of Gaugler et al. (1990) by examining nematode survival after 6 weeks of storage at either 10°C or 25°C. The IS5 strain survived significantly better at 25°C than at 10°C (percent survival was 84.3 and 5.3 respectively for I_{30}; and 80.8 vs. 26.6 for I_{25}). On the other hand, HP88 nematodes reared at 25°C survived longer when stored at 10°C than at 25°C (94.7 vs. 47.4%).

DISCUSSION

Our results indicate that the heat tolerance trait in IS5 was preserved for at least 12 generations of rearing under laboratory conditions. Stability of this trait in entomopathogenic nematodes is a significant finding relative to previous studies on genetic improvement. Dunphy and Webster (1986) unsuccessfully attempted to select for temperature tolerance in populations of *H. bacteriophora* (NC strain), and *Steinernema carpocapsae* (Weiser) (Mexican and DD136 strains).

Because the trait stability studies described herein were conducted with and without selection pressure for heat tolerance, the basis of the heat tolerance trait (environmental vs. genetic) was also elucidated. Heat tolerance in IS5 appears to be genetically based but influenced by environmental conditions. If it were based exclusively on environmental factors, then trait stability would have been lost once the selection pressure has been removed and the IS5 nematodes would not have retained heat tolerance after multiple passages at 25°C. Conversely, if the heat tolerance trait was controlled exclusively by genotype without any influence of the environment, no differences between the IS5 populations reared at 25°C and at 30°C would have been observed.

Genetic improvement requires that the population possesses sufficient genetic diversity for the beneficial trait (Glazer et al., 1991). We propose that nematode populations that have been cultured in the laboratory over long periods, such as the commercial strains, may lose the genetic variability necessary for selection of certain beneficial traits. Indeed, Lower et al. (1968) reported increased susceptibility of free living nematodes to selection for heat tolerance in populations with greater genetic variation. Alternatively, low genetic variability may have been present in original isolates due to founder effect. Lack of sufficient genetic variation could have caused the unsuccessful previous attempts to expand thermal ranges in entomopathogenic nematodes which have either failed or been abandoned due to the instability of the selected traits (Burman and Pye, 1980; Dunphy and Webster, 1986). A lack of sufficient genetic diversity in laboratory populations may be overcome by surveying natural populations for desired traits (Gaugler, 1987). The present study supports this premise.

An important avenue of genetic improvement is hybridization. It entails combining beneficial traits from different strains by crosses. Preliminary experiments suggest that the heat tolerance of IS5 can be crossed into the commercial strain HP88. These observations support our identification, based on morphological criteria (data not shown), that the IS5 strain belongs to the species *H. bacteriophora* Poinar.

Various studies have demonstrated that laboratory rearing of biological control agents can result in reduced host finding, fecundity, and longevity (Hopper et al., 1993). The risk of such reductions in fitness may be particularly great for genetically improved organisms that are exposed to specific selection regimes (Gaugler et al., 1990). In our study, virulence, infectivity, and reproductive potential of the IS5 strain was equal or superior to the commercial HP88 strain. Therefore, it is unlikely that the field efficacy of the IS5 strain will be compromised by successive laboratory reproduction.

Future research will further elucidate the genetics and biochemistry of IS5 and utilize hybridization for genetic improvement.

ACKNOWLEDGEMENTS

David I. Shapiro gratefully acknowledges support from the United States - Israel Education Foundation. This research was funded in part by the Binational Agricultural Research Development Fund, grant No. IS-2099-92C, and by the French - Israeli Cooperation in Agricultural Biotechnology project No. 4434.

REFERENCES

BURMAN, M., & PYE, A. E. 1980. *Neoaplectana carpocapsae*: movements of nematode populations on a thermal gradient. Exp. Parasitol. 49, 258-265.

DUNPHY, G. B., & WEBSTER, J. M. 1986. Temperature effects on the growth and virulence of *Steinernema feltiae* strains and Heterorhabditis heliothidis. J. Nematol. 18, 270-272.

GAUGLER, R. 1987. Entomogenous nematodes and their prospects for genetic improvement. In "Biotechnology in Invertebrate Pathology and

Cell Culture" (K. Maramorosch, Ed.), pp. 457- 484. Academic Press, San Diego, CA.

GAUGLER, R., CAMPBELL, J. F., & MCGUIRE, T. R. 1990. Fitness of a genetically improved entomopathogenic nematode. J. Invertebr. Pathol. 56, 106-116.

GLAZER, I., GAUGLER, R., & SEGAL, D. 1991. Genetics of the nematode *Heterorhabditis bacteriophora* strain HP88: the diversity of beneficial traits. J. Nematol. 23, 324-333.

HOPPER, K. R., ROUSH, R. T., & POWELL, W. 1993. Management of genetics of biological-control introductions. Annu. Rev. Entomol. 38, 27-51.

KAYA, H. K., & GAUGLER, R. 1993. Entomopathogenic nematodes. Annu. Rev. Entomol. 38, 181-206.

KOLTAI, H., GLAZER, I., &. SEGAL, D. 1994. Phenotypic and genetic analysis of two new mutants of *Heterorhabditis bacteriphora*. J. Nematology 26, 32-32.

LOWER, W. R., HANSEN, E., & YARWOOD, E. A. 1968. Selection for adaptation to increased temperatures in free-living nematodes. Life Sci. 7, 139-146.

MAULEON, H., BRIAND, S., LAUMOND, C., & BONIFASSI, E. 1993. Utilisation d'enzymes digestive pour l'etude du parasitisme des Steinernema and et des Heterorhabditis envers les larves d'insectes. Fundam. Appl. Nematol. 16, 185-186.

SHAPIRO, D., GLAZER, I., & SEGAL, D. Trait stability and storage capacity in the heat tolerant entomopathogenic nematode *Heherorhebditis bacteriophora* (IS5). Biol. Control (in press)

WOODRING, J. L., & KAYA, H. K. 1988. Steinernematid and heterorhabditid nematodes: a handbook of techniques. Southern Cooperative Series Bulletin, 331. 30 pages.

ZIONI (COHEN-NISSAN), S., GLAZER, I., & SEGAL, D. 1992. Phenotypic and genetic analysis of a mutant of *Heterorhabditis bacteriphora* Strain HP 88. J. Nematology 24, 359-364.

STARVATION AND PERSISTENCE OF *HETERORHABDITIS* SP.

Paul Fitters and Christine Griffin
St Patrick's College
Maynooth, Ireland

Introduction

One of the major limitations of *Heterorhabditis* spp., which are more infective for certain targets than *Steinernema* spp., is their poor shelf life. In the absence of a host, either in soil or in storage, the non feeding infective stage of parasitic nematodes uses up its stored energy reserves (Tiilikkala, 1992). Depletion of food reserves, such as lipids, is likely to restrict their shelf life. It has been suggested that decline of lipid levels is largely dependent on locomotory activity (Wallace, 1965; Reversat, 1981). Vänninnen (1990) has shown that a decrease of lipid contents below 10% (measured as stained area) leads to weakened mobility and ability to infect insects. The question addressed in this paper is whether a low level of activity is an important trait for long term storage.
Activity levels, assessed on a range of North west European (NWE) *Heterorhabditis* isolates of different geographic origin, were measured as mobility in sand columns and in water. A new method for measuring starvation rate of nematodes is introduced and compared to a chemical lipid analysis.

Material and methods

Nine NWE isolates of *Heterorhabditis* were cultured on the greater wax moth (*Galleria mellonella*) at 20°C. The infective juveniles were concentrated to 5000 per Petri dish (approximately 1000 nematodes/ml), and incubated at 20°C in the dark for up to seven weeks and measurements were made at weekly or two weekly intervals. The experiment was repeated three times and the results are the mean of the three experiments.

Rate of **depletion of stored reserves**, was measured with the use of a Magiscan image analyzer. Nematodes become visibly lighter during starvation, due to the depletion of both lipids and other components. Optical density of 40 heat killed nematodes per isolate were measured after 0, 1, 2, 3, 5 and 7 weeks. At the same time intervals the level of extractable **triglycerides** was assessed using a commercial assay kit from Sigma (GPO-Trinder). Prior to this assessment all the nematode samples were frozen until used simultaneously. Triglycerides were released by sonicating the nematodes on ice. Chloroform and methanol was used to separate lipids from other nematode substances. Subsequently, the triglycerides were digested enzymatically and the resulting glycerol was coloured specifically and determined by spectrophotometry. The **survival rate** was assessed by counting the number of nematodes alive in each Petri dish.

Mobility in sand columns was assessed in the first week. PVC-columns (20 cm long) were filled with silver sand with a water content of 2.3% (w/w). The columns were made up of 5 rings, each containing approximately 65 gr of sand. For each isolate, 5000 nematodes/200 µl tap water are added to the top ring of each of 3 columns. The columns were closed, top and bottom with parafilm. After 24 hours at 20°C in the dark, the top and bottom ring were taken apart and the nematodes in them were extracted and counted. Mobility was calculated as number of nematodes recovered from the bottom ring divided by the number recovered from the top ring.

Mobility in water was measured in the first week, by counting the number of head movements nematodes make during 30 seconds. The nematodes were placed in a watch glass under a binocular with top light and left for at least 1 hour to acclimatise. The temperature was kept at 20°C (± 1°). For each block, head movements of 30 nematodes per isolate were registered.

Results and discussion

There was good correlation (R^2 = 0.874) between the optical densities measurements and the chemical triglyceride assessment over a 7 weeks starvation period. The method for measuring starvation rate based on optical densities was easier to use, gave instant results, required fewer nematodes and the variance in the results was smaller than with the chemical triglyceride assessment. Therefore we conclude that density measurements of nematodes are a good alternative to measurements of extracted triglycerides.

Starvation rate assessments, measured as nematode density and as triglyceride levels, showed that isolate EU 17 uses up its energy reserves significantly more slowly than the other eight isolates. Due to differences in size, the initial lipid levels of the isolates were not the same. However when size was taken into account, by dividing nematode density by nematode area, the slow depletion of energy reserves of isolate EU 17 was shown more clearly (fig 1).

Persistence of the nematodes showed a typical S-curve. After 4 weeks the number of nematodes alive started to decrease. After 7 weeks the number of nematodes alive dropped dramatically below 50% for all isolates, but remained above 80% for EU17. After 14 weeks storage, 38% of the EU17 nematodes were still alive compared to less than 10% for the other isolates.
A 50% mortality for all isolates was found at an area integrated density level of 0.38 (± 0.02).

Significant differences were found between isolates for the mobility in water. EU 17, EU 103 and UK 211 made less head movements per minute in water than the other isolates. No significant differences were found for the mobility in sand columns, but EU 17 was the least mobile of all isolates.
The mobility of EU17 in sand columns and in water was found to be lower than that of most of the other isolates This suggests that its slower rate of utilisation of reserves is due to its lower activity rate. Its better persistence in water may

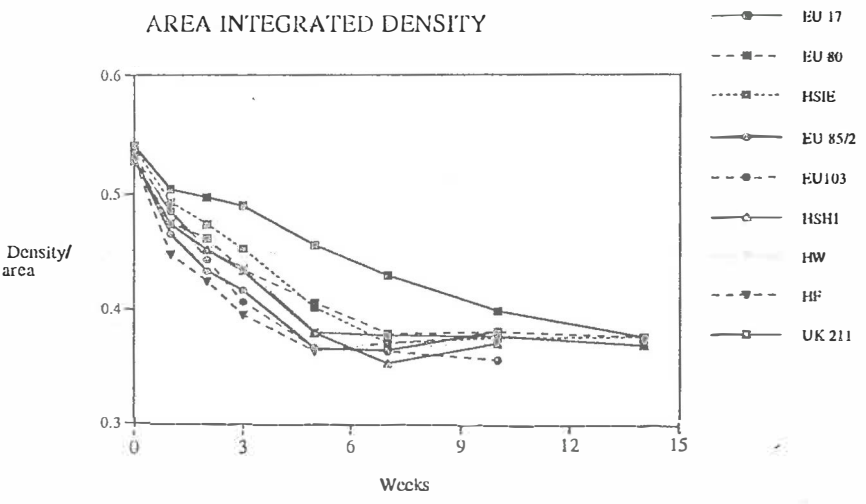


Figure 1: Area integrated density (nematode density / nematode area) of 9 North-West European *Heterorhabditis spp.* over time in water at 20°C.

in turn be due to these traits. However, the other isolates did not differ greatly from each other, and when comparing all nine strains, no significant correlations between mobility and lipid utilisation were found.

Although a relationship was found between persistence and starvation, activity levels at start of storage were not found to be related to persistence.

In conclusion, we think that isolate EU17 is a potentially useful source of genetic information for the improvement of *Heterorhabditis* spp.

Acknowledgement

We like to thank Eric Meijer (IPO-DLO, Wageningen, The Netherlands) for making the programme used for measuring nematode densities on the image analyzer. This work was carried out with the financial support of the EU (ERBCHBCT930486).

References

Reversat, G. (1981). Consumption of food reserves by starved second stage juveniles of *Meloidogyne naasi* under conditions inducing osmobiosis. Nematologica 27, 207-214.

Tiilikkala, K. A. (1992). Influence of soil temperature on initial energy reserves of *Globodera rostochiesis* larvae. Fundamental Applied Nematology 15 (1), 49-54.

Vänninnen, I. (1990). Depletion of endogenous lipid reserves in *Steinernema feltiae* and *Heterorhabditis bactiophora* and effect on infectivity. Proc. Vth Int. Coll. Invertebr. Pathol., p.232. Adelaide, Australia, 20-24 August 1990.

Wallace, H. R. (1968). Dynamics of nematode movement. Annual Review of Phytopathology 6, 91-114.

How to enhance the plating efficiency of *Photorhabdus luminescens*

Karina C. Krasomil-Osterfeld[1],
Bill Donavan[2]
and Karl-Hermann Osterfeld[1]

[1]ECOGEN-Bio Germany, Klausdorferstr. 28-36, 24223 Raisdorf; Germany;
[2]ECOGEN Inc. 2005 Cabot Bvd. West, Langhorne, PA 19047-1810, USA

Introduction

Photorhabdus luminescens, the gram-negative bacterium symbiotically associated with entomopathogenic nematodes of the genus *Heterorhabditis* is known to be very susceptible to physiological changes triggered by environmental factors (Krasomil-Osterfeld, 1995). The mechanisms involved are not yet fully understood, and under *in vitro* culture conditions the complexity of the reaction pattern is mostly experienced as high variability in experiments realised with this bacterium.

Plating efficiency is an example for this variability. In literature, plating efficiency of *Xenorhabdus* spp., the symbiont of *Steinernematid* nematodes, is often described to be very poor (Nealson *et al.*, 1990). To increase plating efficiency several recommendations were published: Poinar & Thomas (1967) suggested to avoid change of medium and to use bacteria that were cultured for only 8 h in peptone water. Changes in temperature and salinity during dilution and plating were responsible for low plating efficiency due to Götz *et al.* (1981). Xu & Hurlbert (1990) gave the advice to avoid exposure of media to daylight or light from fluorescent lamps because traces of radicals were produced and are suspected to inhibit the growth of *Xenorhabdus* cells.

We now suggest a new method that has been proven to be very effective in increasing the plating efficiency of *Photorhabdus* cultures: add sterile filtered culture supernatant to the *Photorhabdus* cells before plating.

Material and Methods

Photorhabdus luminescens, isolated from *Heterorhabditis bacteriophora* (New Jersey strain RS92-M1), stored at -80°C, was cultured in 100 ml shake flasks filled with 20 ml Lab Lemco broth (LL) at 25°C and after 24 h or 48 h the culture broth was centrifuged at 12000 g for 2 minutes. Supernatant was collected and sterile filtered (0,2 µm filter), bacterial pellets were washed (3x) and resuspended. Serial dilutions were made in 1:10 steps. The medium for washing, resuspending and diluting was LL for one part of the pellets, and supernatant for the other part of the pellets.
Cells were counted in the 10^{-2} dilution and dilutions 10^{-3} to 10^{-7} were plated to LL-agar plates (1,5% agar) by the droplet method: 5 drops (10µl each) were placed to the agar in a row at 1 cm distance, then plates were inclined so that the drops spread in a line on the agar surface without reaching the Petri dish border.

Plates were incubated at 25°C, colonies were counted after 48 h under a binocular and plating efficiencies were expressed as counted colony forming units in percent of the expected cell number.
In further experiments the supernatant was heated to 100°C for 10 minutes, or the supernatant was treated with Proteinase K and supernatant concentrations were varied by mixing pure supernatant with fresh medium (1%, 10% and 30% supernatant) and using this mixture for washing, resuspending and diluting the cells.

Results

In eight repetitions of this experiment a striking difference in plating efficiency was found. Table 1 summarises these findings for an average experiment.

Table 1. Influence of dilution medium and culture time on plating efficiency of *Photorhabdus luminescens*

Dilution Medium	24 h culture			48 h culture		
	Plating efficiency at approx. cells per plate			Plating efficiency at approx. cells per plate		
	1.000	100	10	2.000	200	20
LL broth	0	0	0	16%	11%	0
supernatant	47%	66%	89%	33%	68%	68%

Cells from 24 h culture were almost completely inhibited from forming colonies, when diluted with fresh medium, while they showed high recovery even at the highest dilutions, when diluted with supernatant.
Cells from 48 h culture showed the same high plating efficiency when treated with supernatant, however, the variant diluted with fresh medium showed a greater plating efficiency than in the 24 h experiment.

Supernatant heated to 100°C reduced plating efficiencies as much as fresh medium. Treatment of supernatant with Proteinase K resulted in plating efficiencies as high as in the variants with untreated supernatant. Different supernatant concentrations had a great impact on plating efficiency: 30% supernatant increased plating efficiency, while 10% and 1% supernatant gave plating efficiencies comparable to cells diluted in fresh medium (Table 2).

Table 2. Plating efficiency of *Photorhabdus luminescens* cells at different supernatant concentrations (24 h

Supernatant concentration	Plating efficiency at approx. cells per plate			
	60.000	6.000	600	60
0%	0.01%	0.02%	0	0
1%	0.01%	0	0.1%	0
10%	0.003%	0.05%	0	0
30%	n.c.[1)]	9%	15%	8%
100%	n.c.[1)]	n.c.[1)]	75%	72%

[1)] n.c., not countable, colony density too high

Discussion

The results of these experiments have demonstrated that a supernatant factor is able to enhance plating efficiency significantly. The described method provides a useful tool to make plating experiments with *P. luminescens* more effective and reliable. But it also can initiate studies to elucidate the mechanisms of these phenomena that indicate that cell-to-cell communication must be involved. The observed effect resembles in some aspects density-dependent signalling and autoinduction phenomena known from other gram-negative bacteria. (Bainton *et al.*, 1992; Fuqua *et al.*, 1994; Boettcher & Ruby, 1995).

However, if the observed effect is interpreted as autoinduction of cell division (as required for colony formation), it must be growth phase dependent, because otherwise no culture could ever start from the normally low bacterial inoculation densities given by single nematodes that invade the host insect. The difference in plating efficiency in late exponential growth phase (24 h culture) and stationary growth phase (48 h culture) too suggests that the observed suppression of colony formation by fresh medium is growth phase dependent.

Further experiments are desirable to characterise the factor in the supernatant responsible for the enhanced plating efficiency. Its role in the symbiotic life cycle should also be investigated.

References

Bainton, N. J., Bycroft, B. W., Chhabra, S. R., Stead, P., Gledhill, L., Hill, P. J., Rees, C. E. D., Winson, M. K., Salmond, G. P. C., Steward, G. S. A. B., Williams, P. 1992. A general role for the *lux* autoinducer in bacterial cell signalling: control of antibiotic biosynthesis in *Erwinia*. Gene **116**, 87-91.

Boettcher; K. J. & Ruby, E. G. 1995. Detection and quantification of *Vibrio fischeri* autoinducer from symbiotic squid light organs. J. Bacteriol. **177**, 1053-1058.

Fuqua, W. C., Winans, S. C., Greenberg, E.P. 1994. Quorum sensing in bacteria: the LuxR-Lux-I family of cell density-responsive transcriptional regulators. J. Bacteriol. **176,** 269-275:

Götz, P., Boman A. & Boman, H. G. 1981. Interactions between insect immunity and an insect-pathogenic nematode with symbiotic bacteria. Proc. Roy. Soc. Lond., **212**, 333-350.

Krasomil-Osterfeld, K. C. 1995. Influence of osmolarity on phase shift of *Photorhabdus luminescens*. Appl. Environ. Microbiol., **61**, 3748-3749.

Nealson, K. H., Schmidt, T. M. & Bleakley, B. 1990. Physiology and biochemistry of *Xenorhabdus*. *In*: Gaugler, R. and Kaya, H. K. (Eds.): Entomopathogenic nematodes in biological control, Boca Raton, Fla., CRC Press, 271-299.

Poinar, G. O. Jr. & Thomas, G. M. 1967. The nature of *Achromobacter nematophilus* as an insect pathogen. J. Invertebr. Pathol., **9**, 510-514.

Xu, J. & Hurlbert, R. E. 1990. Toxicity of irradiated media for *Xenorhabdus* spp. Appl. Environ. Microbiol., **56**, 815-818.

VIRULENCE FACTORS IN ENTOMOGENOUS RHABDITID NEMATODES: PROTECTIVE IMMUNITY AGAINST *XENORHABDUS NEMATOPHILUS* AND ANTIBACTERIAL ACTIVITY IN IMMUNOLOGICALLY RESPONSIBLE HOST, *GALLERIA MELLONELLA*

JAROSZ J., STEFANIAK M., JABŁOŃSKI P.

Department of Insect Pathology. Marie Curie-Skłodowska University. Akademicka 19, 20-033 Lublin, Poland

Abstract. In larvae of *Galleria mellonella,* LPS *Escherichia coli* induced antibacterial activity of lysozyme and cecropin-like proteins that protected well the insect from fatal *Pseudomonas aeruginosa* septicaemia but not from *Xenorhabdus nematophilus* infections. The immune response of the cecropin-like type can not be provoked in larvae exposed to the nematode juveniles though every injection of non-self bodies and a cuticle injury could induce in *Galleria* the hypersynthesis of haemolymph lysozyme and the *de novo* synthesis of cecropin-like immune proteins. Antibacterial assays have shown that *S. carpocapsae* affects during parasitism an immune response in *Galleria*. It can be evidenced by total reduction of bactericidal activity of cecropin-like molecules in immunologically responsible host. Proteinases released into larval body during parasitism with *S. carpocapsae* and in insects injected intrahaemocoelically with phase 1 variants of *X. nematophilus* alone, depress specifically the antibacterial activity of cecropin-like immune system. It partly explained the question why immunologically responsible host does not withstand the infection with *X. nematophilus*.

Introduction

Insect parasitic rhabditid nematodes, *Steinernema* and *Heterorhabditis*, are mutualistically associated with nematophilic bacteria and have biologically intricate mode of pathogenesis. After entering an insect host, infective juveniles move to the hemocoel where they release symbiotic bacteria. The bacteria multiply rapidly in the insect blood, and the host dies usually on day 2. The rhabditid plays the role of a vector for associated bacteria that are believed to be responsible for the host's death (Poinar and Thomas, 1966; Thomas and Poinar, 1979). A specific association between entomogenous rhabditid *Steinernema carpocapsae* and its symbiotic bacterium *Xenorhabdus nematophilus* contributes, therefore, to virulence of this insect parasite.

Bacterial associates occur in two dimorphic forms, the phase 1 and phase 2 variant (Akhurst, 1980; Akhurst and Boemare, 1990), that differ morphologically on Tergitol-7 agar and to some extent biochemically. There is no sufficient evidence to explain the role of these two variants in the killing of the parasitized host. The nematode/bacterial complex and phase 1 variant of bacterial associate produce during parasitism an extracellular proteinase(s) that depresses specifically an immune response in insect to bacterial infections (Jarosz, 1987; Jarosz, Boemare and Gaj, 1994) . The destruction of cecropin-like antibacterial proteins by proteinase produced by *S. carpocapsae* was previously reported by Götz et al. (1981). Inhibitory effect on insect inducible cell-free antibacterial immunity of the immune inhibitor of the type A (InA) produced by *Bacillus thuringiensis* (Sidén et al., 1979) that represents a specific type of proteolytic activity, is already documented (Dalhammar and Steiner, 1984). The inhibitor degrades proteolytically the cecropin-like polypeptides in immunized insect, a group of basic, small molecular weight antibacterial factors generated in insects by bacterial infections. Cecropins seem to be primary immune proteins responsible for elimination of bacterial invaders from insect hemocoel (Jarosz, 1995).

This article will attempt to demonstrate whether invasion by entomogenous rhabditid nematode *S. carpocapsae* could affect the cell-free immune response in the greater wax moth larvae, and whether inducible non-self antibacterial immunity of the host coincides with the protective immunity against *X. nematophilus*. If is true that the invasion with *S. carpocapsae* can depress the immune

response in the host insect, and the immunologically responsible host does not withstand the infection with *X. nematophilus*, the concept that exoproteinase(s) released during parasitism represents a virulence factor of the *S. carpocapsae* would be considered to be correct. This is a part of an ongoing investigation to define the factors responsible for the virulence of nematode/bacterial complex of *S. carpocapsae*.

Material and Methods

Nematode and isolation of bacterial associates. The Polish indigenous strain of the rhabditid *Steinernema carpocapsae* (strain PLNc82) was isolated originally from a soil sample using the „*Galleria* trap" technique of Bedding and Akhurst (1975). Invasive nematodes reared according to the method of Dutky et al. (1964) on larval greater wax moth *Galleria mellonella*, were harvested from host cadavers and immediately used for invasion. Phase 1 variants of *X. nematophilus* were isolated by homogenizing surface-sterilized infective juveniles (Akhurst, 1980), and phase 2 colonies from larval plasma of *G. mellonella* parasitized with *S. carpocapsae*. All isolates of *X. nematophilus* were routinely checked for pure cultures by inoculation into 7th-instar larvae, determination of pathogenicity and symptoms of septicaemia. Only isolates (strain XnN3 and XnN4) of a high virulence (LD_{50} less than 30 cells per 7th instar of *Galleria* when introduced intrahaemocoelically) were used for experimental infections.

Insect host, immunization and antibacterial assays. Last-instar larvae of *G. mellonella* were obtained from laboratory cultures, and had been reared on dark honey drawn combs at 29°C in total darkness. For immunization, batches of 18 larvae were injected into the abdominal haemocoel with LPS of *Escherichia coli*, LPS of *Pseudomonas aeruginosa* or with heat-killed cells of *P. aeruginosa*. Haemolymph collected from each specimen was tested for antibacterial activity. Another set of insects were exposed to an invasion with *S. carpocapsae/X. nematophilus* complex and antibacterial activity of haemolymph was detected whithin 24 h after an invasion. Antibacterial activity of cecropin-like proteins was recorded as the diameter of the inhibition zone around wells (diameter 2,7 mm) in thin agar plates seeded with *Escherichia coli* D31 (Hoffmann et al., 1981). Lysozyme activity in haemolymph samples was also detected in an inhibition zone assay using freeze-dried *Micrococcus luteus* incorporated into Sörensen buffer (pH 6,4) solidified with agarose (0,7%), according to Mohrig and Messner (1968). Different dilutions of cecropin A (Sigma) from *Hyalophora cecropia* and chicken egg-white lysozyme were used as standards.

Extracellular proteinase and inhibition test for cecropin antibacterial activity. Larval extracts containing crude samples of exoproteinase were prepared from *G. mellonella* killed by *S. carpocapsae*/bacterial complex or by *X. nematophilus* alone. Usually ten larvae were thoroughly blended in 10 ml of distilled water. The obtained homogenates were centrifuged to separate the tissue debris and cold-sterilized by passage through a Schot G-5 filter. A total activity of extracellular proteinases in larval extract was quantified by the conventional agar-diffusion assay technique and expressed in the terms of trypsin activity (EC. 3.4.4.4).

As an *in vitro* test for the presence of proteinase that depresses specifically the bactericidal activity of cecropins, the ability of larval extract to inactivate the lysis of *E. coli* D31 caused by immune haemolymphs of *G. mellonella* or *Celerio euphorbiae,* was determinated. Reaction mixture contained 25µl of immune haemolymph, 10; 15; 20; 25; 30; 35; 40; 45 or 50 µl of proteinase sample and sterile water up to 100 µl in an Eppendorf tube. The remaining activity of cecropins in assay mixture was tested against *E. coli* D31 after 30 min of pre-incubation at 23°C. Maximal inhibitory dilution (MID) was defined as the amount of proteinase contained in greatest dilution of larval extract that completely inactivated the antibacterial activity of cecropin-like factors in 25µl of insect immune haemolymph.

Protective immunity. The number of live bacteria (*Pseudomonas aeruginosa,* a pyocyanin-producing strain H3, or *Xenorhabdus nematophilus* strains XnN3 and XnN4) in different samples were determinated in a viable count assay on agar plates with nutrient broth. Within 24 h post-

immunization, larvae of *G. mellonella* were challenged with lethal doses of bacterial parasite by injecting 5µl of a cell suspension (about 300 bacterial cells/larva) into the abdomen. The control unvaccinated insects taken from the stock culture, were also challenged with the lethal dose of the pathogens. The onset of disease was then observed and mortality due to *Pseudomonas* or *Xenorhabdus* septicaemia was recorded daily. The protective immunity (100 minus percent mortality) was calculated from the cumulative mortality on day 2.

Resuits and Discussion

Insects have effective immune systems composed of both haemocytic and humoral components that normally protect the insect body cavity from bacterial infections. Cellular immune reactions include phagocytosis, nodule formation and encapsulation (Salt, 1970; Ratcliffe and Rowley, 1979; Götz and Boman, 1985). Humoral immune reactions employ an arsenal of defeces to successfully combat a diversity of non-self bodies, including bacterial pathogens. They involve synthesis and release of several antibacterial immune proteins, some capable of killing both gram-positive and gram-negative bacteria (Boman and Hultmark, 1987). There are at least three major classes of immune proteins in holometabolous insects: the lysozyme, cecropins and attacins that are responsible for antibacterial immunity. Following the bacterial infection or injection of an immunizing agent, there is a rapid and selective synthesis of effector antibacterial substances, and a maximum level is reached over a time period which coincides with the maximum *in vivo* induced protective immunity against *Pseudomonas aeruginosa* and *in vitro* antibacterial activity (Jarosz, 1979; Chadwick and Aston, 1991).

Lysozyme, a naturally occurring protein found in haemolymph of most insects increases greatly during an immune response (Mohrig and Messner, 1968; Jarosz and Śpiewak, 1979). Inducible immunity appears after induction with bacteria or non-living objects which disturb the physiological state of an insect. In lepidopterans, the aquired cell-free immunity is associated with the *de novo* synthesis of cecropins (Boman and Hultmark, 1987) and attacins (Hultmark et al., 1983). Cecropins represent a family of closely-related, strongly basic proteins with a molecular weight of 4kD which have a potent antibacterial activity. They are most important in the defence both against gram-positive and gram-negative bacterial invaders (Jarosz, 1995).

Antibacterial activities in larvae of *G. mellonella* parasitized with *S. carpocapsae* is shown in Table 1. Larvae were exposed continuously to infective juveniles of the nematode and the antibacterial activity of lysozyme and cecropin-like type was recorded 24 hours post-exposure.Haemolymph from unparasitized larvae contains an innate titer of lysozyme but no activity

Table 1. Haemolymph antibacterial activity in larvae of *Galleria mellonella* parasitized with infective juveniles of *Steinernema carpocapsae*

Specimens	Antibacterial activity (µg/ml)*			
	Lysozyme		Cecropins	
	Range	Mean value (± SD)	Range	Mean value (± SD)
Steinernema carpocapsae	120.2-1122.0	627.1 (372.1)	0	0
Unparasitized	21.9-645.7	404.2 (194.9)	0	0

*Lysozyme activity given in µg/ml of haemolymph is expressed in the term of chicken egg-white lysozyme activity (E.C. 3.2.1.17).

of cecropin-like proteins could be detected in the haemolymph of all individuals tested. Although there are noticed considerable differences in the antibacterial activity of different individuals, it is clear that lysozyme titer increases demonstrably in *Galleria* larvae parasitized with nematode juveniles of *S. carpocapsae*. Interestingly, the immune response of the cecropin-like type is not provoked in larvae exposed to the nematode (Table 1) though every injection of non-self bodies and

a cuticle injury could induce in *Galleria* the hypersynthesis of haemolymph lysozyme and the *de novo* synthesis of cecropin-like molecules (Mohrig and Messner, 1968; Jarosz, 1979, 1995; Jarosz and Śpiewak, 1979).

From a series of experiments where larvae of *Galleria* were immunized with LPS *E. coli* and 18 hours later, when an immune response developed in insect, they were parasitized with *S. carpocapsae* it is obvious that this insect obligatory parasite depresses an immune response of the host (Table 2). Such strong inductor of antibacterial immunity as LPS *E. coli* induced bactericidal activity of cecropin immune proteins and markedly increased (5-fold) the titer of insect lysozyme in haemolymph inspected within 36 h post-immunization. It unequivocally indicates that LPS *E. coli* provoked the *Galleria* immune system to produce more antibacterial activity in order to eliminate an infection. Surprisingly, the antibacterial activity began to decrease in immunologically responsible host that then was parasitized with *S. carpocapsae*. The lysozyme titer dropped from 2052 μg found in LPS *E. coli*-injected larvae to 1644 μg/ml in vaccinated insects but then exposed to the nematode juveniles. No trace of cecropin antibacterial activity was detected within 36 hour post-vaccination in larvae infected by *S. carpocapsae* (see Table 2). Animals at the time of antibacterial assays were fully vigorous, but on day 2 they perished due to *X. nematophilus* septicaemia.

Table 2. Depressive effects on antibacterial immune response of *Steinernema carpocapsae* in larvae of *Galleria mellonella* vaccinated with LPS *Escherichia coli*

Specimens	Antibacterial activity (μg/ml)*			
	Lysozyme		Cecropins	
	Range	Mean value (±SD)	Range	Mean value (±SD)
Immunized with LPS *E. coli*	1122.0-6025.6	2052.2 (1317.3)	3.1-263.0	37.2 (44.0)
Immunized but then parasitized with *S. carpocapsae*	851.1-3467.4	1644.2 (707.7)	0	0

* Antibacterial activity of the cecropin-like immune proteins is calculated as the activity of cecropin A (Sigma) from *Hyalophora cecropia*.

From a series of recent studies (Stephens, 1962; Mohrig and Messner, 1968; Jarosz, 1979), it is known that the antibacterial activity in cell-free haemolymph of the greater wax moth following intracoelomic injection of *Pseudomonas aeruginosa* vaccines is correlated with the protective immunity of the larvae. Under the above circumstances, it seemed necessary to ascertain whether an immunologically responsible host is also protected against infections with *X. nematophilus*, the bacterium intimately associated with the nematode *S. carpocapsae*.

As shown in Table 2 and in Fig. 1, the immunizing agent (LPS *E.* coli) enhanced the antibacterial activity of larval haemolymph and well protected the *G. mellonella* against fatal *P. aeruginosa* septicaemia, but not against *X. nematophilus*. In general, the decrease in the titre of antibacterial activity of cecropin-like factors in immunized *Galleria*, and then parasitized by juveniles of *S. carpocapsae*, coincided with the lack of protective immunity of the larvae against *X. nematophilus*. In the group of larvae immunized with LPS *P. aeruginosa*, only 24% of animals infected with 330 cells of *X. nematophilus* (strain XnN3) survived the challeging dose of bacterial parasite but as much as 85% of individuals withstood the lethal dose of *P. aeruginosa* and metamorphosed normally to adults. Moreover, practically any larva of *Galleria* vaccinated with *Pseudomonas* does not ensure immunity when more virulent strain XnN4 was used as a challenging bacterium. For comparison, almost 80% of larvae immunized with *P. aeruginosa* vaccine survived the infection with *P. aeruginosa*. Bacteriologic broth induced antibacterial immunity in *Galleria* and protected effectivelly the larvae from fatal septicaemia of *P. aeruginosa* (Mohrig and Messner, 1968; Jarosz, 1979), but it not protected the insects against infections with *X. nematophilus* (see Fig. 1).

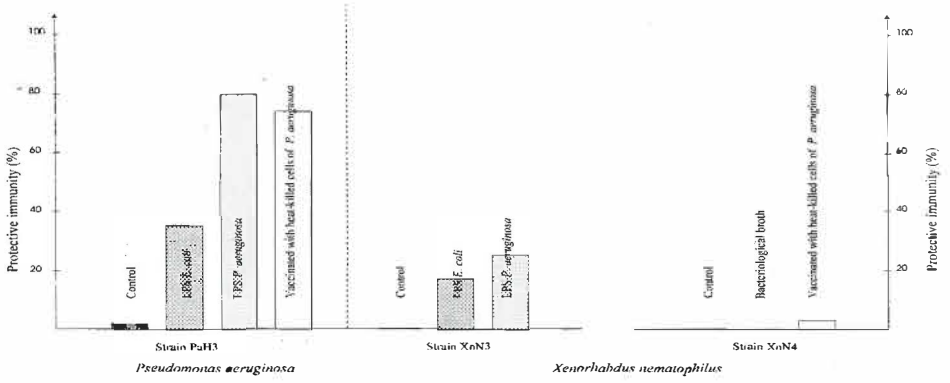


Fig.1. Protective immunity against *Pseudomonas aeruginosa* and *Xenorhabdus nematophilus* in larvae of *Galleria mellonella* vaccinated with LPS *E. coli* , LPS *P. aeruginosa* or heat-killed cells of *P. aeruginosa* .

As can be seen in Table 3, the extracellular proteinase released into larval body during *S. carpocapsae* parasitism specifically depresses cecropin-like antibacterial activity of insect immune haemolymph. The *in vitro* assay system demonstrated that cecropins of *Celerio euphorbiae* were

Table 3. Maximal inhibitory dilution for larval extracts depressing *in vitro* bactericidal cecropin-like activity of lepidopterous insects

Proteinase source	Proteolytic activity (μg/ml)*	Maximal Inhibitory Dilution (percent of larval extract)	
		Galleria mellonella	*Celerio euphorbiae*
Steinernema carpocapsae	1.2	20	15
Xenorhabdus nematophilus (phase 1)	0.7	50	40

*Expressed in the terms of trypsin activity (E.C. 3.4.4.4).

more susceptible to an inhibitory effect of proteinase than were *Galleria* cecropins. It is obvious from MID values determined for larval extracts from *Galleria* killed by *S. carpocapsae/X. nematophilus* complex or by phase 1 of *X. nematophilus* that the ability of an inhibitor to inactivate the antibacterial activity of cecropin-like polypeptides depends on the concentration of the proteinase to which immune haemolymph was exposed. Extracts from unparasitized larvae failed to induce any inhibitory effects.

It is postulated that *S. carpocapsae* proteinase, interfering with the inducible antibacterial immunity of insects, resembles an immune inhibitor of the type A (InA) from *B. thuringiensis* (Edlund et al., 1976; Sidén et al., 1979). It is reasonable to conclude that by neutralizing the antibacterial activity of cecropins in haemolymph of the host to which *X. nematophilus* is sensitive, the proteinase could contribute to the overall virulence of *S. carpocapsae*. It partly explains the question why immunologically responsible host is not protected against infections with *X. nematophilus*.

References

AKHURST R.J., 1980. Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. J. Gen.Microbiol., 121: 303-309.

AKHURST R.J. & BOEMARE N., 1990. Biology and taxonomy of *Xenorhabdus*. In: *Entomopathogenic Nematodes in Biological Control*, (eds. R.R. Gaugler, H.K. Kaya), 75-90, CRC Press, Inc., Boca Raton, Fla.

BOMAN H.G. & HULTMARK D., 1987. Cell-free immunity in insects. Ann. Rev. Microbiol., 41: 103-126.

BEDDING R.A. & AKHURST R.J., 1975. A simple technique for the detetction of insect parasitic rhabditoid nematodes in soil. Nematologica, 21: 109-110.
CHADWICK J.S. & ASTON W.P., 1991. Antibacterial immunity in *Lepidoptera*. In: *Immunology of Insects and Other Arthropods*, (ed. A.P. Gupta), 347-370, CRC Press, Boca Raton, Ann-Arbor, London.
DALHAMMAR G. & STEINER H., 1984. Characterisation of inhibitor A, a protease from *Bacillus thuringiensis* which degrades attacins and cecropins, two classes of antibacterial proteins in insects. Eur. J. Biochem., 139: 247-252.
DUTKY S.R. THOMPSON J.V. & CANTWELL G.E., 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol., 6: 417-422.
EDLUND T., SIDÉN I. & BOMAN H.G., 1976. Evidence for the immune inhibitors from *Bacillus thuringiensis* interfering with the humoral defence system of saturniid pupae. Infect. Immun., 14: 934-941.
GÖTZ P. & BOMAN H.G. 1985. Insect immunity. In: *Comprehensive Insect Physiology Biochemistry and Pharmacology*, (eds. G. A. Kerkut, L. I. Gilbert), Vol. 3: 453-485, Oxford/New York: Pergamon, 625 pp.
GÖTZ P., BOMAN A. & BOMAN H.G., 1981. Interactions between insect immunity and an insect-pathogenic nematode with symbiotic bacteria. Proc. R. Soc., London, 212B: 333-350.
HOFFMANN D., HULTMARK D. & BOMAN H.G., 1981. Insect immunity: *Galleria mallonella* and other lepidoptera have cecropia-P9-like factors active against gram negative bacteria. Insect Biochem., 11: 537-548.
HULTMARK D., ENGSTRÖM A., ANDERSSON K., STEINER H., BENNICH H. & BOMAN H.G., 1983. Insect immunity. Attacins, a family of antibacterial proteins from *Hyalophora cecropia*. EMBO J., 2: 571-576.
JAROSZ J., 1979. Simultaneous induction of protective immunity and selective synthesis of haemolymph lysozyme protein in larvae of *Galleria mellonella*. Biol. Zentralbl., 98: 459-471.
JAROSZ J., 1987. Immune inhibitor of cecropin-like activity from cadavers of the greater wax moth larvae parasitized with *Heterorhabditis bacteriophora*. Society for Invertebrate Pathology XX Annual Meeting, University of Florida, Gainesville, 81-82.
JAROSZ J., 1995. Haemolymph immune proteins protect the insect body cavity from invading bacteria. Comp. Biochem. Physiol. (In press)
JAROSZ J., BOEMARE N. & GAJ C., 1994. The anti-cecropins agent contributes to insecticidal nature of *Heterorhabditis bacteriophora*. VIth International Colloquium on Invertebrate Pathology and Microbial Control, Montpellier, 261-263.
JAROSZ J. & ŚPIEWAK N., 1979. Comparative levels of lysozyme activity in larvae and pupae of *Galleria mellonella* after various particulate and soluble materials injection. Cytobios, 26: 203-219.
MOHRIG W. & MESSNER B., 1968. Immunreaktionen bei Insekten. I. Lysozym als grundlegender antibakterieller Faktor im humoralen Abwehrmechanismus der Insekten. Biol. Zentralbl., 87: 439-470.
POINAR G.O. & THOMAS G.M., 1966. Significance of *Achromobacter nematophilus* Poinar and Thomas (*Achromobacteriacae: Eubacteriales*) in the development of the nematode DD-136 (*Neoaplectana* sp., *Steinernematidae*). Parasitology, 56: 385-390.
RATCLIFFE N.A. & ROWLEY A.F., 1979. Role of hemocytes in defence against biological agents. In: *Insect Hemocytes*, (ed. A.P. Gupta), 331-414, London: Cambridge Univ. Press, 614 pp.
SALT G., 1970. The cellular defence reactions of insects. Monogr. Exp. Biol., Vol. 16. London: Cambridge Univ. Press, 118 pp.
SIDÉN I., DALHAMMAR G., TELANDER B., BOMAN H.G. & SOMERVILLE H., 1979. Virulence factors in *Bacillus thuringiensis*: Purification and properties of a protein inhibitor of immunity in insects. J. Gen. Microbiol., 114: 45-52.
STEPHENS J. M., 1962. Bactericidal activity of the blood of actively immunized wax moth larvae. Can. J. Microbiol., 8: 491-499.
THOMAS G.M. & POINAR G.O., 1979. *Xenorhabdus* gen. nov. a genus of entomopathogenic, nematophilic bacteria of the family *Enterobacteriacae*. Int. J. Syst. Bacteriol.,29: 352-360.

EFFECT OF BIOTIC FACTORS ON CONTROL OF *SITONA LINEATUS* BY NEMATODES

M. Jaworska and D. Ropek

Department of Agricultural Environment Protection, Academy of Agriculture, Mickiewicz Ave., 21, 31-120 Cracow, Poland

An important pest species for legume crops is the pea and bean weevil *Sitona lineatus* L. Although the adult weevils feed on the leaves of the plants, their larvae do the most damage feeding on the root surface. Entomopathogenic nematodes (EN) have gained attention as potential control agents in IPM programmes to legume crops for soil-inhabiting insects like *S. lineatus*.

Tests in our laboratory, carried out on *S. lineatus* adults and other *Sitona* species coming from clover stands, showed susceptibility of these weevils to EN.

The objective of this paper was to record the effects of different insect stages od *S. lineatus* and different cultivars of host plant on susceptibility of insects for nematode infection and usefulness for nematode multiplication.

Under continuous contact all insect stages reared on early pea were highly susceptible to infection by EN. Nematodes multiplied within larvae, pupae and adults but the best inside pupae.

Survival and activity of *S. lineatus* weevils during the egg laying period may be connected with host plant preference. Preference of pea cultivars influenced the low feeding activity and low survival, the higher susceptibility to entomogenous nematodes and the lower reproduction in comparison to those from beans.

Introduction

In ecological agriculture, legumes are important not only for the production of protein rich foliage, but also for the fixation of nitrogen in the soil.

Pea and bean are very prone to pests and diseases. An important pest species is the pea and bean weevil *Sitona lineatus* L. The adult weevils make characteristic U-shaped notches on leaf margins. Most of the damage to the plants results from larval feeding on the roots and root nodules, which reduces nitrogen fixation.

Entomopathogenic nematodes (EN) have gained attention as potential control agents in IPM programs to legume crops for such soil - inhabiting insects like *S. lineatus.*

Tests in our laboratory on *Sitona* coming from clover stands (Wiech and Jaworska 1990) showed that these weevils are susceptible to EN.

The objective of this study was to determine the effects of different stages of insect and plant hosts on the susceptibility of *S. lineatus* to infection by EN.

Methods

To determine effect of insect stages and host-plant on susceptibility and usefulness for nematode multiplication, early and late instar of *Sitona* larvae pupae and young weevils were collected from soil and plants of various cultivars (cv) of bean (*Vicia faba maior* L.), field bean (*Vicia faba minor* L.) or pea (*Pisum sativum* L.) and field pea (*Pisum arvense* L.). The following cv were used; early bean ,,White Windsor", late bean ,,White Handgan", late field bean ,,Nadwiślański", early pea ,,Sześciotygodniowy", late pea ,,Nike" and field pea ,,Fidelia" (Jaworska, 1992).

The survivability of pea weevils coming from the legumes was determined by putting them into small glass jars with moistened sand and host plants and rearing for 1 month in the dark at 23°.

Nematode infection and multiplication was determined by placing 300 IJ of *Steinernema carpocapsae*, *S. feltiae* and *H. bacteriophora* in each of 10 cm-d of sterile

Petri dish lined with Whatman filter papers. Experiments were carried out at 28°C in the dark.

Data were subjected to statistical analysis, for cumulative mortality after Freeman-Tukey transformation, by analysis of variance and means were separated according to Duncan's multiple-range test.

Results

Pea weevil adults collected and reared on various cultivars of annual legumens survived from 95 % in the first week to about 10 % after the month. Weevils collected from bean were alive longer. Larvae and adults of *S. lineatus* reared on early pea were highly susceptible to infection by EN. Pupae were less susceptible (natural mortality was 40 %), although they were the best host among studied stages.

S. lineatus adults were high susceptible to all entomogenous nematodes under continuous contact. Differences in infection rates depended on nematode species and the host plant of *S. lineatus* adults.

All three nematodes multiplied inside weevil bodies. For *S. carpocapsae* and *H. bacteriophora,* the best host was adult coming from early bean. Ineffective juveniles of nematodes emerged in greater quantities from weevils reared on early bean or late field bean in comparison with weevils reared on early field pea.

Discussion

The effect of pea and bean cultivars on susceptibility of *S. lineatus* larvae to EN has been demonstrated previously (Jaworska and Ropek, 1994). This study showed that activity and natural mortality of weevils, after their egg laying period. may be connected with preference of their host plant. The most attractive plants were early field pea or early pea. During the egg laying period the preference changed (Jaworska, 1992). Early or late bean

were more suitable hosts, probably because they provided better conditions for egg laying and adults survival. Preference of early field pea and other pea cultivars influenced the low feeding activity of *S. lineatus* and the lower average body weight, the higher susceptibility of entomogenous nematodes, and the lower reproduction. In presence of the same entomogenous nematodes adults of *S. lineatus* were more susceptible to pathogens in comparison to weevils coming from white and red clover (Wiech and Jaworska, 1990). It can be expected that the preference of legumes plants influences survivability and susceptibility of weevils to pathogens.

Conclusions

Results indicate the importance to biological control of including different insect stages and different host-plants in testing nematodes against an insect species.

References

Jaworska, M., 1992, Über den Befall einjähriger Leguminosen durch den Erbsenrüssler, *Sitona lineatus* L. (*Col. Curculionidae*). Anz. Schädling Pflanzensch Umweltschutz 65, 70-72.

Jaworska, M., and Ropek, D., 1994, Influence of host-plant on the susceptibility of *Sitona lineatus* L. (*Col. Curculionidae*) to *Steinernema carpocapsae* Weiser. Journal of Invertebrate Pathology, 64, 96-99.

Wiech, K., and Jaworska, M., 1990, Susceptibility of *Sitona* weevils (*Col. Curculionidae*) to entomogenous nematodes. J. Appl. Ent., 110, 214-216.

DETOXICATION OF NEMATODES

M. Jaworska[1], A. Gorczyca[1], A. Szeliga[2], J. Sepioł[2] and P. Tomasik[2]

[1] Department of Agricultural Environment Protection, Academy of Agricultural, Mickiewicz Ave., 21, 31-120 Cracow, Poland

[2] Department of Chemistry, Academy of Agricultural, Mickiewicz Ave., 21, 31-120 Cracow, Poland

Howewer, generally, the entomopathogenic nematodes under laboratory study exhibited a strong resistance to the metal ions expressed in terms of the mortality within the 96 hour laboratory tests.

In spite of a lack of acute toxicity of most of the single metal ions in 96 hour laboratory tests, these ions affected the infectivity of the entomopathogenic nematodes.

The 96 hour laboratory tests were carried out in order to prove possible synergistic and antagonistic interactions between pairs of metal ions. Thus, the pairs of ions were arranged from each of Al, Cd, Co (II), Cr (III), Cr (VI), Cu (II), Fe (III), Li, Mo (VI), Ni (II), Se (IV), V (V) and Zn on one side and Mn (II) and Mg on other. The mortality of *S. carpocapsae* and *H. bacteriophora* caused by the most toxic Pb (II) ions could significantly be reduced by an addition of Mn (II) or Mg (II) ions. Mn (II) and Mg ions considerably inhibited the nematodes and inducted the nematode infectivity against *Galleria mellonella* caterpillars.

Introduction

In our recent papers the toxicity of single metal ions againts *Steinernema carpocapsae* (Jaworska et all., 1995a) and *Heterorhabditis bacteriophora* (Jaworska et all., 1995b) nematodes has been presented. These studies have revealed a different biological response of both genera to the same metal ions administered under identical conditions. However, generally, the entomopathogenic nematodes under laboratory study axhibited a strong resistance to the metal ions expressed in terms of the mortality within the 96 hour laboratory tests. An enhanced toxicity from Cu (II), Zn and especially Pb (II) ions was observed at concentrations only randomly met in the environment. The infectivity of the nematodes was also affected even in the presence of the majority of seemingly nontoxic metal ions.

Following our recent studies on the metal-metal interactions we have developed studies on the metal-metal interactions in the title nematodes. The results of the studies are presented in this paper.

Materials and methods

The aqueous solutions were prepared (the concentrations in mg/L are given in parentheses) of analitycal grade $Al_2(SO_4)_3 \cdot 18\ H_2O$ (100); $CdCl_2$ (150); $CoCl_2$ (70); $Cr_2(SO_4)_3 \cdot 18\ H_2O$ (70); K_2CrO_4 (70); $CuSO_4 \cdot 5\ H_2O$ (150); $FeCl_3$ (400); $Li_2SO_4 \cdot H_2O$ (40); $MgSO_4 \cdot 7\ H_2O$ (320); $MnSO_4 \cdot 5\ H_2O$ (800); SeO_2 (11); $(NH_4)_6Mo_7O_{24} \cdot 6\ H_2O$ (200); $NiSO_4 \cdot 6\ H_2O$ (100); $Pb(NO_3)_2$ (400); NH_4VO_3 (105) and $ZnCl_2$ (400). These solutions were twice diluted either with water for the toxicity tests of single metal ions or with solutions of Mn (II) respectively (100, 200, 400) or Mg salt (32, 64, 160).

Control samples were in Petri dishes with 3 small test tubes filled with 1 cm^3 of one of above aqueous solution and containing aproximately 3 x 30 infective juveniles of either *Steinernema carpocapsae*, Polish local strain or *Heterorhabditis bacteriophora* (Poinar), both cultivated on *Galleria mellonella* caterpillars. Similar Petri dishes containing approximately the same number of infective juveniles of above generae were filled with 1

cm^3 of solutions prepared by blending of above solutions with either Mn (II) or Mg salt solutions. Observations of the nematode mortality and activity was conducted at 25°C in 12 hour intervals over the course of 96 hours using a stereomicroscope. The ability of metal ion - treated nematodes to cause host mortality was tested againts last - instar larvae of *G. mellonella*.

Results

The mortality dynamic of *S. carpocapsae* and *H. bacteriophora* nematodes intoxicated with Pb (II) was simultaneously inhibited by increasing concentrations of Mn (II) and Mg salt solutions. The inhibitions effect in particular metal ion pairs was dependent to a certain on concentration of either the Mn (II) or Mg additive. Particularly strong inhibition was observed in the case of all combinations of Pb (II), with Mn (II) as well as Mg. It means that the mortality of *S. carpocapsae* and *H. bacteriophora* caused by the most toxic Pb (II) ions could significantly be reduced by an addition of either Mn (II) or Mg. The detoxifying effect in *S. carpocapsae* and *H. bacteriophora* due to antagonism between Cd, Co (II), Cr (VI), Ni (II), Pb (II), V (V) as well as Zn ions on one hand and Mn (II) as well as Mg ions on the other, are represented. Not only the mortality was reduced but also the infectivity of nematodes towards *G. mellonella* was essentially and, sometimes fully, retained. Even in the case of intoxication of the larvae with Pb (II) which resulted in full mortality, the intervention of Mn (II) ions protected these organisms and lifted the infectivity up to 60 % of that in control. This was the sole example of the stronger inhibition from Mn (II) ions. In contrast to this the inhibition from Mg ions was more effective in cooperation with all Cd, Cr (VI) and Ni (II). Both Mn (II) and Mg were equally effective in pairs with Co (II), V (V) and Zn.

Conclusions

1. The mortality of *S. carpocapsae* and *H. bacteriophora* caused by the most toxic Pb (II) ions could significantly be reduced by an addition of Mn (II) or Mg (II) ions.
2. The intervention of Mn (II) or Mg (II) ions protected the nematodes and lifted their infectivity.

References

Jaworska, M., Sepioł, J., Tomasik, P., 1995a, Effect of metal ions on *Steinernema carpocapsae* entomopathogenic nematodes., Water, Air, Soil Pollutions., in the press.

Jaworska, M., Gorczyca A., Sepioł, J., Tomasik, P., 1995b, Effect of metal ions on *Heterorhabditis bacteriophora* entomopathogenic nematodes., ibid., in the press.

Yields and infectivity of a tropical steinernematid cultured *in vivo* and *in vitro*

[1]MICHELLE M. FINNEGAN, [2]CHAERANI, [1]MARTIN J. DOWNES & [1]CHRISTINE T. GRIFFIN.

[1]Department of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland
[2]Department of Phytopathology, BORIF, Bogor, Indonesia.

Introduction

Entomopathogenic nematodes (*Steinernema* spp and *Heterorhabdiitis* spp.) have considerable potential for the control of several important insect pests. Because of the warm, humid conditions, tropical countries are ideally suited to the use of these nematodes. As part of an ongoing programme of research in Indonesia, entomopathogenic nematodes of both genera were isolated widely. We here report some preliminary experiments on methods of culturing an indigenous *Steinernema* ; the isolate used is one of two RFLP types of *Steinernema* isolated in the archipelago, and cannot be assigned to any currently described species (Reid, pers. comm.). The nematodes were cultured using standard *in vivo* (Dutky *et al.*, 1964) and *in vitro* (Bedding 1981) methods at 25, 28 and 30°C. The nematodes produced by the various media at the three temperatures were counted and their infectivity for *Galleria mellonella* tested.

Materials & Methods

Nematodes : *Steinernema* sp. INA S14, a strain of an undescribed species, was isolated in the Moluccas, Indonesia.

Production : Nematodes were produced *in vivo*, based on the method of Dutky *et al.* (1964), in each of two species of insect, and *in vitro*, using Bedding's (1981) flask method. The *in vitro* medium (polyether polyurethane foam coated with 80% chicken offal homogenate and 20% water) was inoculated with the natural bacterial symbiont of INA S14. Petri dishes containing 20 larvae of either *Galleria mellonella* or *Tenebrio molitor* of known weight, and flasks containing 25 gm of chicken offal medium, were each inoculated with 2,000 infective juveniles (IJs) of INA S14. Dishes and flasks were incubated at 25, 28 and 30°C. There were 5 replicates per medium / temperature combination.

Nematodes emerging from insects were harvested daily and stored at their culture temperature. When emergence ceased, harvests within any given replicate were pooled, washed and counted. Flask cultures were harvested two weeks post nematode inoculation by placing the foam contents into a mesh net submerged in water, overnight. Infective juveniles emerging were washed and counted.

Infectivity: The infectivity of nematodes produced under each set of conditions was tested : 1,000 infective juveniles were exposed to 10 larvae of *G. mellonella* in a Petri dish (3 replicates / treatment). Following incubation at 28°C for 3 days, insects were dissected and the number of first generation adult nematodes was taken as representative of the number of infective juveniles that had entered.

Results

In both flasks and *G. mellonella*, numbers of IJs produced decreased with rising temperature (Figs 1A & 2A) and, in flasks, the differences between temperatures were significant. In infectivity tests with IJs produced by these two methods, the numbers of IJs infecting at 28°C increased with culture temperature, although only the lowest culture temperature (25°C) was significantly different from the rest in each case (Figs 1B & 2B). These trends were not observed when nematodes were cultured in *T. molitor* larvae; there was little difference between culture temperatures in terms of yield (Fig 3A), and the most infective nematodes produced from *T. molitor* were those that had been cultured at 28°C, the intermediate temperature (Fig. 3B).

For both insect species, the time to first nematode emergence decreased with increasing temperature; at 28 and 25°C IJs began to emerge 11 days after infection while at 30°C they first emerged after 8 days. IJs began to emerge earlier from *G. mellonella* than from *T. molitor*, and emerged over a longer period (4-5 days for *G. mellonella* compared to 3 -4 days for *T. molitor*) .

Overall, the nature of the medium used did not affect the yield of IJs/gm of medium ($P>0.05$). The average yield was 1.1 $x10^5$ IJs /g medium. However, the nature of the medium used did affect infectivity: IJs produced in either *G. mellonella* or *T. molitor* were more infective than those produced *in vitro* ($P<0.05$). Of all the nine medium/temperature combinations, the most infective IJs were those produced at 28°C in *T. molitor* and at 30°C in *G. mellonella*.

Conclusions & Discussion

The Indonesian isolate INA S14 of the tropical species of *Steinernema* can be cultured successfully in the larvae of *G. mellonella* or *T. molitor* and *in vitro* in "Bedding flasks", and with an overall yield of 10^5 IJs/g medium. This yield is relatively low for entomopathogenic nematodes in general, but is similar to that obtained by Bedding (1981) for *S. glaseri*, a species which INA S14 resembles in having a large IJ (unpublished data). Those IJs produced in insects were more infective than those produced in flasks. This is in line with findings of other workers that the nature of the culture medium used affects the quality of infective juveniles produced (Alikhan *et al.*, 1985; Gaugler & Georgis, 1991).

Nematodes produced at lower temperatures in both *G. mellonella* and flasks were both more abundant and of lower infectivity than those produced at higher temperatures. Among the possible explanations for this inverse relationship between yield and quality are the following :

1) culturing time for nematodes is shorter at 30°C, leaving the harvested IJs older at the time of testing, and their greater maturity may account for their higher infectivity (Griffin, 1995). However, if this were the case, the same phenomenon should also be observed for IJs produced in *T. moitor*.

2) because fewer infective juveniles are produced at the higher temperatures there is more substrate available per nematode, which may result in their quality and infective ability being greater that that of IJs produced in high numbers where medium is more scarce.

Although differences were not significant, there is evidence that 28°C was optimum for yield in *T. molitor*, matching the 28°C significant optimum culture tempertaure for infectivity. Differences between the two insect species in the timing of nematode emergence and the way in which yields and infectivity are affected by temperature may be accounted for either in terms of direct effects such as quality of the insect tissues, or indirectly by their different insect sizes and susceptibilities resulting in different nematode population dynamics. Zervos *et al.* (1991), for example, showed how inoculum size, interacting with temperature, affected time to first emergence, duration of emergence and yields of IJs in *G. mellonella.*

Acknowledgement

This work was carried out with the financial support of the EU (TS3*-CT92-0018).

References

Alikhan, M.A., Bednarek, A and Grabiec. S. (1985). The physiological and morphological characteristics of *Neoplectana carpocapsae* (Nematoda, Steinernematidae) in two insect hosts. J. Invertebr. Pathol. 45, 168-173.

Bedding, R.A. (1981). Low cost *in vitro* mass production of *Neoplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. Nematologica 27, 109-114.

Dutky, S.R., Thompson, J.V., and Cantwell, G.E. (1964). A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol. 6, 417-422.

Gaugler,R. and Georgis, R. (1991). Culture method and efficacy of entomopathogenic nematodes (Rhabditida: *Steinernematidae* and *Heterorhabditidae*). Biol. Contr. 1, 269-274.

Griffin, C.T. (1995). Effects of prior storage conditions on the infectivity of *Heterorhabditis* sp. (Nematoda : Heterorhabditidae). Fundam. appl. Nematol. (in press).

Zervos, S., Johnson, S.C., & Webster,J.M. (1990). Effect of temperature and inoculum size on reproduction and development of *Heterorhabditis heliothidis* and *Steinernema glaseri* (Nematoda : Rhabditoidea) in *Galleria mellonella*. Can. J. Zool. 69 : 1261-1264.

Fig. 1 In Vitro

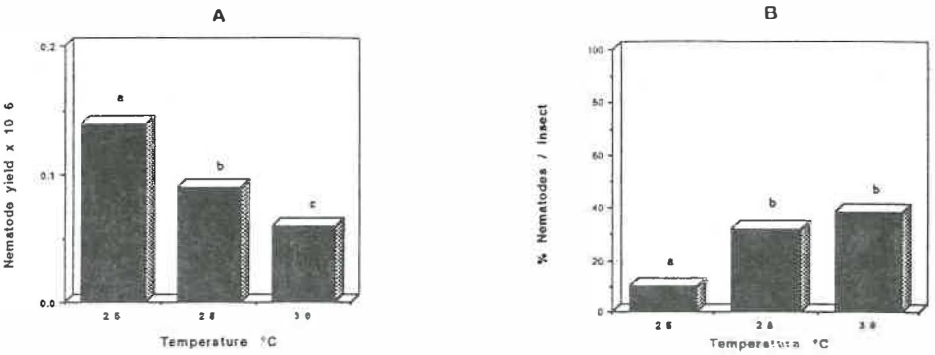


Fig. 2 Galleria

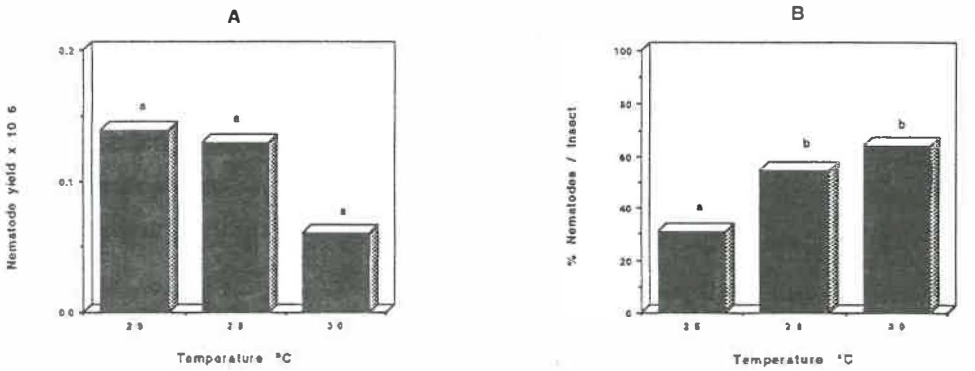
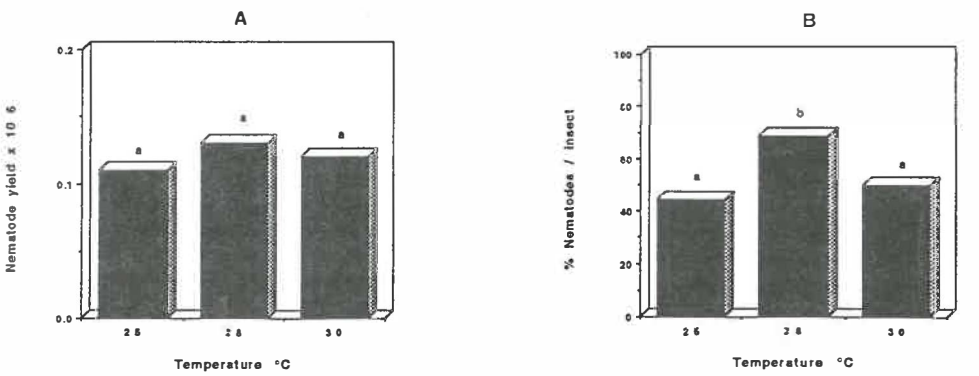


Fig.3 Tenebrio



Figs. 1-3. Number of infective juveniles (x10^6) of *Steinernema* sp. INA S14 produced / gm of medium at 25, 28 and 30°C (Figs 1A, 2A, 3A), and their subsequent infectivity for *Galleria mellonella* larvae at 28°C (Figs 1B, 2B, 3B). Nematodes produced on chicken offal (Fig. 1), *Galleria mellonella* (Fig. 2) and *Tenebrio molitor* (Fig. 3). Columns (within each group) accompanied by the same letter do not differ significantly (ANOVA followed by Duncans multiple range test; $P < 0.05$)

Compatibility of *Steinernema carpocapsae* with pesticides.

B. Barbarossa, R. Frulla, G. Grazzi, L. Rovesti.

C.N.R. - Centro di Studio dei Fitofarmaci
Bologna - Italy

Summary

A total of 32 pesticides (13 insecticides, 12 fungicides and 7 herbicides) were tested for their effects on mortality, behaviour and infectivity of the infective juveniles (J3s) of the nematode, *Steinernema carpocapsae*. Results show that J3s are generally very resistant to pesticides, very few of those tested causing more than 10% mortality. Nevertheless, some strongly reduced or almost completely suppressed the movement of the nematode in sand. The latter parameter was also strongly influenced by the testing procedure used (i.e. placement of J3s on top or bottom of the sand columns).

1. Introduction

As for other beneficial organisms, the use of entomopathogenic nematodes in IPM strategies arises questions with regard to their susceptibility to the chemical pesticides possibly used. Within the framework of the activities of the IOBC WG "Pesticides and Beneficial Organisms", a simple method was used to test the effects of several pesticides on the infective juveniles of EPNs. In this paper the final results of the 7th joint testing programme (JTP) are presented. The preliminary results of the 8th testing programme are also briefly discussed.

2.Materials and methods

The pesticides used and the rates tested are indicated in tab. 1. A three-tier test was used to assess the effect of the exposure to the pesticides on the viability, mobility and infectivity of the infective juveniles, as outlined underneath.

Tier 1 (assessment of viability and behaviour)

- J3s were freshly produced in wax moth larvae (*Galleria mellonella*) and stored in water (6°C) in areated flasks for max. 1 week before use in the test.
- Pesticide dilutions (rates as recommended by the WG) were prepared with distilled water. J3s were exposed in Petri dishes (1000 J3s/ml; control J3s in dist. water) for 24 hs.
- viability and behaviour of the J3s were assessed under a stereomicroscope by checking at least 100 nematodes/pesticide.

Tier 2 (assessment of mobility and infectivity)
- After 24 hs. exposure approx. 500 J3s in 0.5 ml of the pesticide were taken from each dish and put on the bottom of a 7 cm high plastic tube (15 replicates/pesticide).
- Tubes were filled with sterilized, moistened sand (sieved through a 35 mesh sieve, moistened with 13% (w/w) distilled water), packed at a density of 1.6 g/cm^3.
- One wax moth larva was placed on top of each sand column, and removed after 24 hs of placing. After removal, larvae from the same treatment (15 for each pesticide tested) were put together in a Petri dish; larval mortality was checked after further 72 hs.
- Negative (only water) and positive (unexposed J3s) controls were included in each test.

Tier 3 (further testing)
- Further tests were carried out as needed (e.g. exposure to lower rates, infectivity in petri dish, etc.) only for those pesticides which significantly affected either viability or mobility/infectivity of the nematodes.

3. Results

On the whole, the infective juveniles of *S. carpocapsae* showed a good tolerance to the tested pesticides. In tab. 1 mortality and parasitism figures for the pesticides tested in the 7th JTP are summarised; only mortality figures are reported for the 8th JTP, as for these pesticides results for mobility/infectivity (sand column test) are still preliminary.

Within the 7th JTP, cyfluthrin and difenoconazole were the most lethal of the pesticides tested, causing in some of the assays up to approx. 40% mortality. However, they had little effect on the parasitization rate as observed in the mobility/infectivity test (sand columns). On the other hand, heptenophos was not as lethal to the J3s (causing less than 10% mortality) but completely inhibited their movement in sand. Additional tests showed that nematodes exposed to this chemical were mostly unable to parasitize the larvae even when placed directly in contact with them. However, they could recover after washing in distilled water, showing that they had retained their infectivity. Heptenophos also inhibited the movement of the nematodes when unexposed J3s were placed in sand treated with a solution of the pesticide (13% w/w). The very low pH of this insecticidal solution could partly account for inactivation of the nematodes, but our results indicate mainly a direct toxicity of this chemical.

As for the 8th JTP, none of the pesticides strongly reduced the viability of the nematodes or the parasitization rate in the sand column test. Nevertheless, some of them clearly altered the behaviour of the nematodes (e.g. mancozeb and abamectine). For this reason, and in the attempt to develop a more sensitive method, all the products for this JTP were also tested placing the nematodes on top of the sand columns. Despite the very erratic response obtained

for untreated nematodes, with this latter method results indicate a much stronger effect for several pesticides (abamectine, mancozeb and carbendazim in particular).

4. Discussion and conclusions

The overall resistance of EPNs to pesticides has been documented by several studies, including those previously carried out in our laboratory (Rovesti et al., 1988; Rovesti and Deseo, 1990; Rovesti and Deseo, 1991). The present results indicate once more the importance of taking into account not only viability, but also mobility/infectivity of the nematodes, when assessing the effects of pesticides. The method used for this study seem to be adequate to show the adverse effects for highly toxic pesticides, but not enough sensitive for not so toxic ones. The high number of J3s used/sand column (500) probably brings about an underestimation of the effect for those pesticides which inhibit nematode movement, in that theoretically only a single nematode is needed to infect and kill the wax moth larva. However, in preliminary trials using 100 and 300 nematodes, the parasitization rate for unexposed J3s was never complete or in any case inconsistent using a 24 hour baiting time. Also, the upward movement of the nematodes was initially chosen for our test as it gives more consistent results due to the tendency of this species to disperse upwards (Moyle and Kaya, 1981; Georgis and Poinar, 1983). Though this method provides a much more consistent parasitization rate for untreated nematodes than placement on top of the columns, it appears not to be sensitive enough a method to detect minor reductions in nematode performance.

5. References

GEORGIS, R.& POINAR, G.O.Jr, 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). J. of Nematology, 15:38-311.

MOYLE, P.L. & KAYA, H.K., 1981. Dispersal and infectivity of the entomogenous nematode, *Neoaplectana carpocapsae* Weiser (Rhabditidae: Steinernematidae), in sand. J. of Nematology, 13: 295-300.

ROVESTI, L., HEINZPETER, E.W., TAGLIENTE, F. & Deseo, K.V., 1988. Compatibility of pesticides with the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae). Nematologica, 34:462-476.

ROVESTI, L.& DESEO, K.V., 1990. Compatibility of chemical pesticides with the entomopathogenic nematodes, *Steinernema carpocapsae* Weiser and *S. feltiae* Filipjev (Nematoda: Steinernematidae). Nematologica, 36: 237-245.

ROVESTI, L.& DESEO, K.V., 1991. Compatibility of pesticides with the entomopathogenic nematode, *Heterorhabditis heliothidis*. Nematologica, 37: 113-116.

Tab. 1 - Pesticides tested and effects on the nematodes.

Active ingredient	Trade mark	Conc. tested (ppm form.)	Conc. tested (ppm a.i.)	pH of solut.	Mean % J3s alive*	Mean % parasitisn.°
7th TESTING PROGRAMME						
B. t. Kurstaki	Delfin WG	1000	-	6.2	100	100
B. t. tenebrionis	Novodor FC	10000	-	5.3	97.8	100
Cyfluthrin	Baythroid 50	500	20	5.4	90.9	100
Cyfluthrin	Baythroid 50	1250	60	5.4	73.6	95.5
Cyproconazole	Alto 100 SL	2500	250	3.8	87.8	100
Difenoconazole	Score EC 250	500	120	6.1	79.3	90
Fluroxypyr	Starane 180	500	900	5.3	88.2	100
Fluvalinate	Klartan	600	140	5.2	99	100
Haloxyfop	Gallant super	3750	390	5.4	94.7	100
Heptenophos	Hostacquick	1000	550	3.6	90.3	22.2
Heptenophos	Hostacquick	1600	800	3.6	91.5	4.4
L-cyhalothrin	Karate	750	30	5.6	95.4	95.5
Lecithin	Bio blatt	1500	380	6.1	86.9	100
Metamitron	Goltix 70 WG	25000	17500	5.3	91.8	100
Penconazole	Omnex WP10	250	20	8.5	94.6	100
Phosmet	Imidan	2500	1250	5.9	92.6	96.7
Tebuconazole	Folicur 250 EC	2500	630	6.1	91.9	100
Teflubenzuron	Nomolt	1000	140	6.4	99.3	97.7
Verticillium lecanii	Micro Germin	4000	-	7.3	97.9	93.3
8th TESTING PROGRAMME						
Abamectine	Vertimec	1000	180	7.4	99.4	
Carbendazim	Bavistin	1000	510	7.5	99.4	
Chloridazon	Pyramin	1200	784	8	98.9	
Cycloxydim	Focus	1200	240	6.6	98.7	
Diafenthiuron	Polo	800	400	7.4	98.4	
Fosethyl	Aliette	2500	1900	3.9	99.6	
Mancozeb	Dithane M45	2000	1200	7.4	99.5	
Mecoprop-p	Duplosan	8000	5000	7.7	99	
Metazachlor	Butisan	1200	600	8	98.8	
Phosalone	Zolone flow	1200	600	8.1	99.2	
Rape seed oil	Telmion	20000	-	6.9	98.6	
Sulphur	Kumulus	4000	3200	7.6	99.2	
Thiophanat-methyl	Topsin M	750	375	7.4	98.4	
Thiram	Pomarsol F	2000	900	7.8	98.7	
Tolylfluanide	Euparen M	1500	750	8.5	100	

* Corrected (Abbott's formula).
° % of G. mellonella larvae parasitised in the sand column test (tier 2).

BIOASSAY FOR THE GENETIC SELECTION OF ENTOMOPATHOGENIC NEMATODES WITH INCREASED PENETRATION ACTIVITY

SULISTYANTO, D., GOTTORF-FOLGERT, I. & EHLERS, R.-U.

Institute for Phytopathology, Dep. Biotechnology & Biological Control, University Kiel, Klausdorfer Str. 28-36, 24223 Raisdorf, Germany

SUMMARY

A bioassay was developed for a genetic selection of entomopathogenic nematodes with increased penetration activity. The results obtained with this assay were compared with those achieved by several selection cycles using the target hosts (*Galleria mellonella* and *Phyllopertha horticola*). The genetic selection could increase the penetration activity of the nematode populations tested: The penetration activity of *H. megidis* (HSH3) into *G. mellonella* increased from 4,8% to 18,4%, compared to 5,3% to 21% in the *in vivo* selection after six and seven selection cycles, respectively. When *P. horticola* was used as host an increase from 5,8 to 17,6% was recorded (*in vivo*: 8,0% to 21,3%) after the same number of selection cycles. Similar results were obtained with *H. bacteriophora*.

INTRODUCTION

Entomopathogenic nematodes, like most other parasites and pathogens, must penetrate through various barries to enter the host's haemocoel and cause an infection. *Steinernema* or *Heterorhabditis* use natural openings (mouth, anus or spiracles) or penetrate directly through the integument (Peters & Ehlers, 1994). Target insect mortality is directly correlated with the number of nematodes inside the haemocoel (Glazer, 1992; Peters & Ehlers, 1994). For this study, the penetration activity is therefore choosen as a target for a genetic improvement of the nematodes's biocontrol potential. A selection process can be carried out using the target host insect. However, this procedure has one major disadvantage. The first individuals to enter the haemocoel are often eliminated by insect defence mechanisms like encapsulation (Peter and Ehlers, 1994) and are thus lost for a genetic improvement. In order to avoid the loss of these individuals an "*in vitro*" penetration bioassay was developed and its potential for a genetic selection was compared with results obtained by an *in vivo* selection process.

MATERIAL AND METHODS

Heterorhabditis bacteriophora, *H. megidis*, *Steinernema feltiae*, *S. glaseri*, and *S. riobravis* were propagated in last instars of the waxmoth *Galleria mellonella*. Dauer juveniles (DJs) had been stored in Ringer's solution at 5°C for not more than 4 weeks.

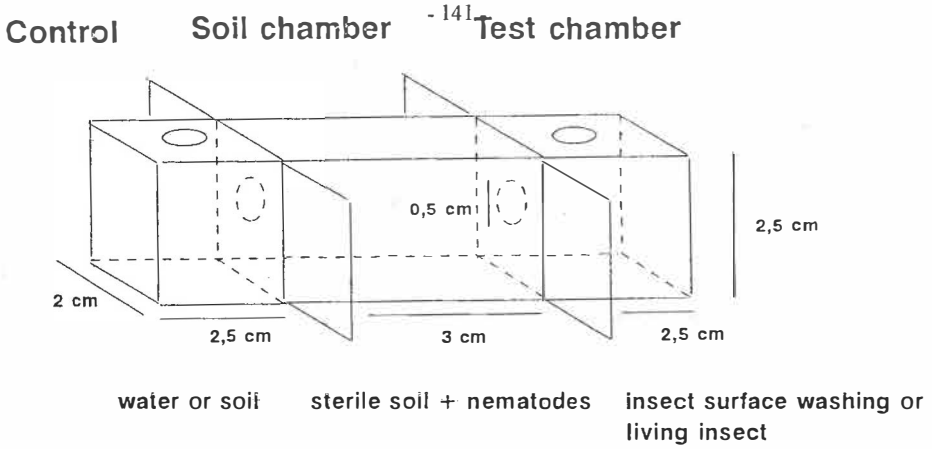


Fig. 1: *In vitro* penetration chamber

The *in vitro* bioassay chamber is shown in Fig. 1. The rectangular chamber filled with sterile soil (15% moisture) and 500 dauer juveniles was attached to two chamber filled with water. The two water chambers were seperated from the sand chamber by a hole (Ø = 0,5 mm) covered with a piece of freshly prepared host insect cuticle of approximately 1 cm². Prior to use the piece of cuticle was rinsed three times in destilled water. Care was taken not to rupture the cuticle and those parts with natural openings (e.g. tracheae) were avoided. One water chamber contained an aquaeuos suspension of surface washings of the host insect: Living larvae were rinsed in destilled water at a ratio of 1:5 for 3 min. and then the water was passed through a Whatman No. 1 filter paper. The chamber was kept at 20°C and 90% relative humidity. After 20 h nematodes which had penetrated the cuticle into the water chambers were counted. Host insects were *G. mellonella*, leather jackets (*Tipula oleracea*) and scarabaeid larvae (*Phyllopertha horticola*). The first two insect species originated from laboratory cultures, the grubs were field collected. For all experiments surface washings and cuticle pieces were from the same insect species.

For a comparison of the penetration activity recorded in the *in vitro* chamber with results obtained with living insects, the chambers were slightly modified. Instead of an insect cuticle nylon mesh of 100 µm was used to cover the holes. The two water chambers were filled with soil and one chamber contained one living host insect larva. After 20 h the insects were removed, washed thoroughly in water and dissected in order to count penetrated DJs.

In a first series of experiments the penetration activity of the different *Steinernema* spp. were compared in the *in vitro* and *in vivo* tests. For each, the *in vitro* and *in vivo* assays, 12 chambers were inoculated with DJs originating from the same propagation batch. The feasibility of a genetic selection was tested with *H. megidis* and *H. bacteriophora*. For the *in vivo* selection 12 insects were used for each host insect species. To evaluate the penetration activity half of the individuals were dissected and the other half was used to propagate the nematodes for the following selection experiment. The *in vitro* selection experiments were carried out

with 12 chambers for each selection step and insect host. Nematodes which had passed through the cuticle were removed and injected into the corresponding host for propagation.

RESULT AND DISCUSSION

All nematode species tested penetrated through the cuticle into the chamber filled with surface washings, whereas in all cases no nematodes were recorded in the control chambers filled with water. This indicates that the cues inducing a penetration behaviour of the dauer juveniles are excreted into the soil surrounding. The first nematodes penetrated 4 h after application into the soil. Some individuals used holes made by their precursors to enter into the water chamber. In order to avoid these individuals in the selection process the duration of the experiments should be reduced.

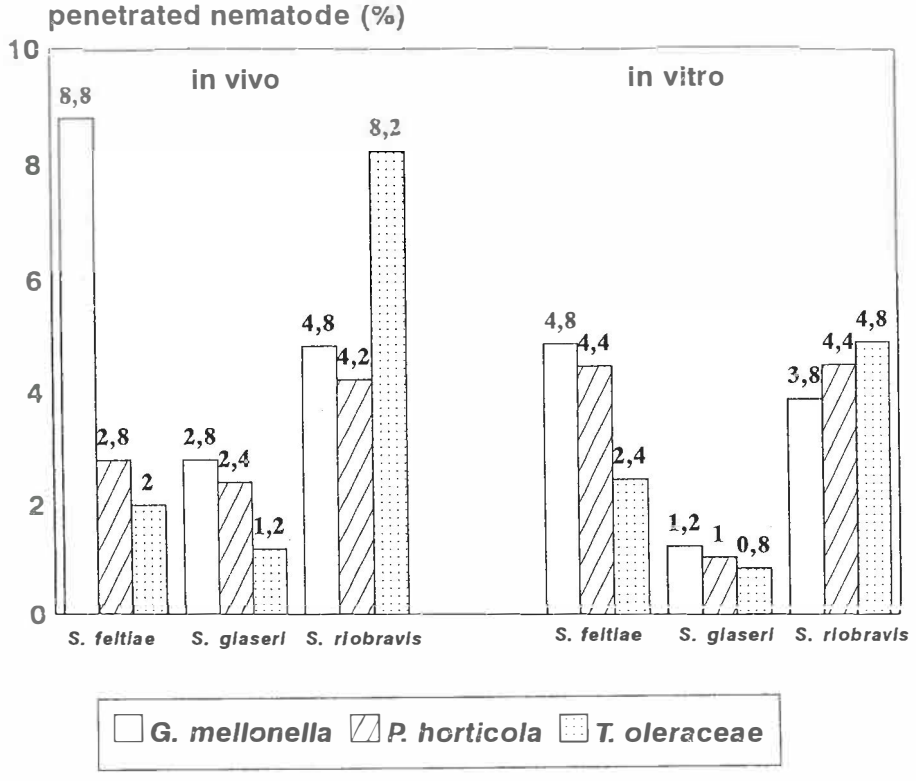


Fig. 2: Penetration activity (% of 500 inoculated nematodes) of *Steinernema* spp. into the host insects or in the chamber containing surface washings of the same host.

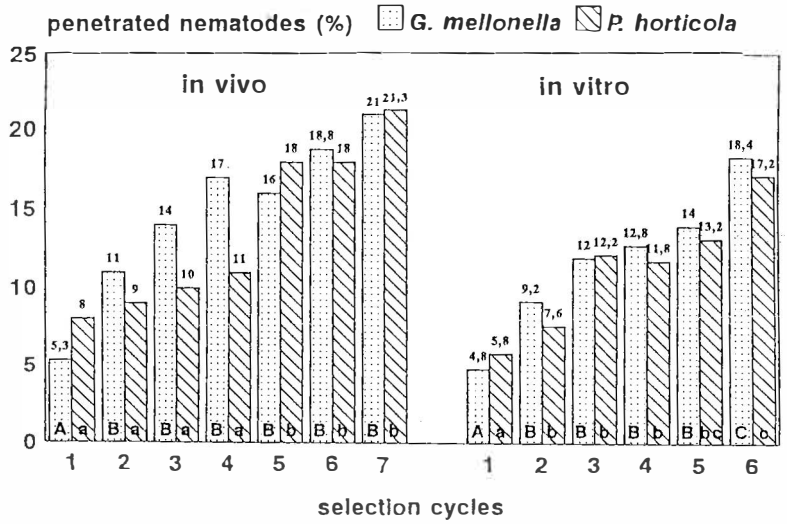


Fig. 3: Percentage of penetrated nematodes (*H. megidis*) recorded during the selection on the natural hosts *G. mellonella* or *P. horticola* (*in vivo*) or in penetration chambers (*in vitro*) Columns followed by the same letter are not significantly different (Chi^2-test, P = 0,05)

The penetration activity in the *in vitro* tests, given as percent of nematodes of the 500 applied to the chambers, was increasing with the concentration of the surface washings (experiment not described). The activity of the different *Steinernema* spp. in both, *in vitro* and *in vivo* tests, differed according to the host insect used (Fig. 2). To draw any conclusions on the host prevalence of the different species from these preliminary results would be too early.

A threefold increase of the penetration activity could be achieved by the genetic selection with *H. megidis* (Fig. 3). Similar results were obtained with *H. bacteriophora*. An increase from 4,0% to 13,8% in the *in vitro* and from 7,2% to 20,4% was recorded in the *in vivo* bioassay with *G.mellonella*. With *P. horticola* the penetration activity increased from 6,6% to 17,6% (*in vitro*) and from 6,4% to 23,6% (*in vivo*). In order to check if the increased penetration activity results in an overall higher control potential, selected nematodes will have to be mass produced and tested under field conditions.

REFERENCES

Glazer, I. (1992). Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematodes to insects. J. Invertebr. Pathol. **59**, 90-94.

Peters & Ehlers, (1994). Susceptibility of leatherjackets (*Tipula paludosa* and *T. oleracea*; Tipulidae: Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. J. Invertebr. Pathol. **63**, 163-171.

DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES IN GRASSLAND HABITAT

J.S.Miduturi, M.Moens and R. Moermans

Research Station for Nematology and Entomology, CLO Brug.Van Gansberghelaan 96, B-9820 Merelbeke, Belgium

SUMMARY

The distribution of entomopathogenic nematodes in a grassland habitat was studied during autumn and winter 1994 using greater wax moth (*Galleria mellonella*) larvae as bait. Infective juveniles that had entered into larvae were extracted by pepsin digestion and counted, over a period of three months. Results indicated that the granulometric composition of the soil with >93% of sand content had large numbers of steinernematids in first sample grid but, it was not strongly pronounced in the second sample grid, whereas *H. megidis* (north-west European (NWE) type) was found when the organic matter content was >7%. In both the sample grids steinernematids had a negative relationship with organic matter content but *H. megidis* (NWE type) had a positive correlation. These results suggests that sand fraction and organic matter composition of the soils can influence the distribution of entomopathogenic nematodes in a natural ecosystem.

INTRODUCTION

During a systematic survey for entomopathogenic nematodes (epn's) in Belgium (West Vlaandren province) we noticed that *Steinernema feltiae* A1 type was predominant followed by *S. affinis*. In only one site was *Heterorhabditis megidis* (NWE type)(Miduturi *et al.*, 1995). Long term persistence and infectivity depend on the redistribution of entomopathogenic nematodes after their application and consecutive cycles. There is little information on the distribution of epn's in their natural habitats. The aim of the present study was to find out the distribution patterns of epn's in a natural ecosystem and relate their distribution to soil factors.

MATERIALS AND METHODS

The sample area had two sample grids, one grid with 66 samples (3 m x 4 m) and the second grid had 48 samples (2 m x 1.5 m). Soil samples were collected at the intersection of each point with a soil auger (10 cm diameter x 10 cm depth) and brought them to the laboratory in plastic bags. Each soil sample was thoroughly mixed and placed in a plastic box (13 x 15 x 3.5 cm) approximately 250 g of soil. Five last instar larvae of *Galleria mellonella* (greater wax moth) were placed in the soil sample, the boxes were covered with Parafilm and kept inverted at 23°C (+ 1°). After 3 days the soil samples were observed and the dead *Galleria* larvae replaced with live ones. The dead *Galleria* larvae were dissected and digested with pepsin, the adult nematodes were counted. Each soil sample had three changes of *Galleria* larvae, until all the infective nematodes were extracted. The nematodes were identified as *Steinernema feltiae* (A1)

and *Heterorhabditis* sp. (NWE type).

The soil samples were analyzed for their organic matter and soil particle analysis with Coulter LS 100 apparatus (fluid model). A particle analysis gave the relative presence of the clay fraction (<4µm), the silt fraction (4-63 µm) and sand fraction (>63µm).

We used distance weighted least squares with an algorithm (McLain, 1974) for three dimensional smoothing. For fitting relationships between numbers of nematodes, and a soil characteristic, we used Levenberg-Marquardt approximation with an algorithm (Press *et al.*, 1987).

RESULTS AND DISCUSSION

In the preliminary distribution studies it was noticed that both *S. feltiae* and *Heterorhabditis* sp. (NWE type) were present in the samples. In the first sample grid only *S. feltiae* was found. The negative exponential model with a surface fitted to the total number of nematodes in each sample site as a function of the sand fraction of the soil, showed large numbers of *S. feltiae* were found in the sites with higher sand fraction (93%). However, low numbers of *Steinemema* were still present in a narrow strip, where the sand fraction decreases to 82% (Fig.1), and the organic matter content had increased.

In the second sample grid there were eight columns and five rows, in first three rows large number of *Heterorhabditis* sp. (NWE type) were found in and the remaining five rows *S. feltiae* were found. In the case of *Heterorhabditis* there is some positive correlation to organic matter content, whereas large numbers of *Steinernema* were found in samples with low organic matter content (4-10%).

The information on the effects of soil on the dispersal and infectivity of epn's has been studied extensively in laboratory conditions. Soil texture particularly clay and silt fractions affected the movement of *S. glaseri* and *S. carpocapsae* (George & Poinar, 1983a and b). *Steinemema feltiae* were found where the sand content was >93% in first sample grid, but they did not show a strong positive relationship to sand in the second sample grid. High organic matter content in soil harbours many soil insects which can be parasitised by epn's. This study showed that in both sample grids where *S. feltiae* was common it had a negative relationship with organic matter content of the soil, while *Heterorhabditis* sp. had a positive relationship.

These are only preliminary results from one habitat. Further studies are needed to confirm these results as well as to study the distribution patterns of epn's.

REFERENCES

George, R. and Poinar, G.J. Jr. (1983a). Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). Journal of Nematology, **15**, 308-311.

George, R. and Poinar, G.J. Jr. (1983b). Effect of soil texture on the distribution and

infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). Journal of Nematology, **15**, 329-332.

McLain, D.H. (1974). Drawing contours from arbitrary data points. Computer Journal, **17**, 318-324.

Miduturi, J.S., M.Moens., and De Grisse A. (1995). Occurrence of entomopathogenic nematodes in West-Vlaanderen province of Belgium. Nematologica, **41**(3), 322 (Abstract).

Press, W.H., Flammery, B.P., Teukolsky, S.A. and Vetterling, W.T. (1987). *Numerical recipes, the art of scientific computing.* Cambridge University press, UK.

INFLUENCE OF DIFFERENT FORMULATIONS ON ENTOMOPATHOGENIC NEMATODE SURVIVAL AND INFECTIVITY

A. NEUMANN & R.-U. EHLERS

Institute for Phytopathology, Dep. Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Klausdorferstr. 28-36, 24223 Raisdorf, Germany

SUMMARY

A dauer juvenile paste was produced at 1,5 Mio. and 1,7 Mio./g wet weight of *H. bacteriophora*. These pastes were mixed with attapulgite and bentonite at 5:6 or with sponge at 2:1. Controls were kept at 500.000 DJ/ml in aerated water. The formulated nematodes were stored at 5°C, 10°C and 24°C. Mortality of the DJs and infectivity against *Galleria mellonella* (bioassay 1:1) was determined over a period of 1 and 2 months.

H. bacteriophora survival and infectivity is higher at lower temperature with no pronounced differences between 5 or 10°C. At 24°C all DJs ussually died after two weeks. No pronounced differences were observed for DJs formulated in attapulgite or bentonite. In sponge and water nematodes survived longer than in clays. Survival and infectivity decreased over the whole testing period. A significant increase in infectivity during the storage time could not be recorded. The results show that non of the tested formulation techniques gave satisfactory survival and nematode quality (infectivity) could not be preserved. The development of improved formulation techniques needs further attention in order to commercialize *H. bacteriophora* on a large scale.

INTRODUCTION

Using large scale bioreactors the mass production of the entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) can be realized at economically reasonable costs (Ehlers, 1995). However, a prerequisite for a successful commercialisation is the development of storage techniques, which can guarantee a shelf life of at least 6 months. Objective of this research was to compare some commercially used formulation compounds at different temperatures.

MATERIAL AND METHODS

Nematodes were provided by Ecogen Bio Germany Gmbh, Raisdorf. They had been produced in liquid culture in a pilot plant bioreactor of 500l volume. After harvesting the dauer juveniles (DJs) from the liquid medium they were transferred onto sieves of 50 μm to separate DJs from other stages and dead nematodes. Only living DJs migrated through the sieve. The sedimented nematodes were afterwards

washed with tap water over another 50 micron sieve to seperate media components and other debris from DJs and then concentrated to a nematode paste of 1.5 Mio. DJs/g for the first and to 1.7 Mio. for the second experiment.

Attapulgite and bentonite clays were dried at 105°C for 24h. The nematode paste was homogeniously mixed with the clays at a ratio of 5:6 and a ratio of 2:1 with sponge cubes of approximately 8 cm^3. The clay-formulated nematodes were transferred to round plastic cups (94 x 25 mm) and covered with a lid. The lid was perforated with 2 little holes in order to allow a gas exchange. The sponge was transferred into 5 l plastic containers. The cups and containers were then stored at 5°C, 10°C and 24°C. For storage in water nematodes were kept in a funnel-shaped continually aerated 1.5 l plastic bottle in 500 g water with 500.000 DJ/g and kept at 5°C only.

Samples were taken one day after formulation and thereafter once a week. The total number of nematodes and the percentage of dead individuals was assassed. A total of 1 g attapulgite/bentonite-nematode mixture was sampled from a minimum of five different areas of the container and transferred to 999 g water. Ten sponge cubes were removed from different parts of the bucket and weighed. They were then transferred to 500 g water and squeezed 20-times. The water suspension was filled up to 500 g and then 1 g was removed and diluted in 999 g water. Out of the dilutions a countable sample was taken for quantification. The differentiation of living and dead DJs was done 1 hour after sampling.

The infectivity of the DJs was assayed on the first and eighth day after formulation and then every second week using last instars of *Galleria mellonella*. A 24 well-plate was filled with one insect larva per well, which was then covered with sand at 15% moisture content. A single DJ per well was then applied on top of the sand. The evaluation of the *G. mellonella* mortality was done 5 days after incubation at 24°C. Only red and bioluminescent insect larvae were counted.

RESULTS

The results on nematode survival and infectivity of the first and second experiment are shown in Fig. 1 and 2, respectively. If the percentage of dead nematodes one day after the formulation is compared between the different techniques applied, it is obvious that a higher amount of nematodes was killed during the process of mixing with the clays than during mixing with sponge. The higher percentage of living individuals in the control in water unline this observation, which was recorded during both experiment.

In all formulations the total number of nematodes decreased with time indicating that dead nematodes were decomposed. The decomposition of dead DJs was slower in sponge than in the clays tested. At a temperature of 24°C hardly any nematodes survived longer than two weeks and their infectivity already declined within the first days of storage. No pronounced differences were recorded between 10° or 5° C for the DJ mortality in the clays. However, in sponge more nematodes survived at the lower temperature. At 5°C the amount of living DJs in attapulgite and bentonite decreased faster than in sponge and aerated water.

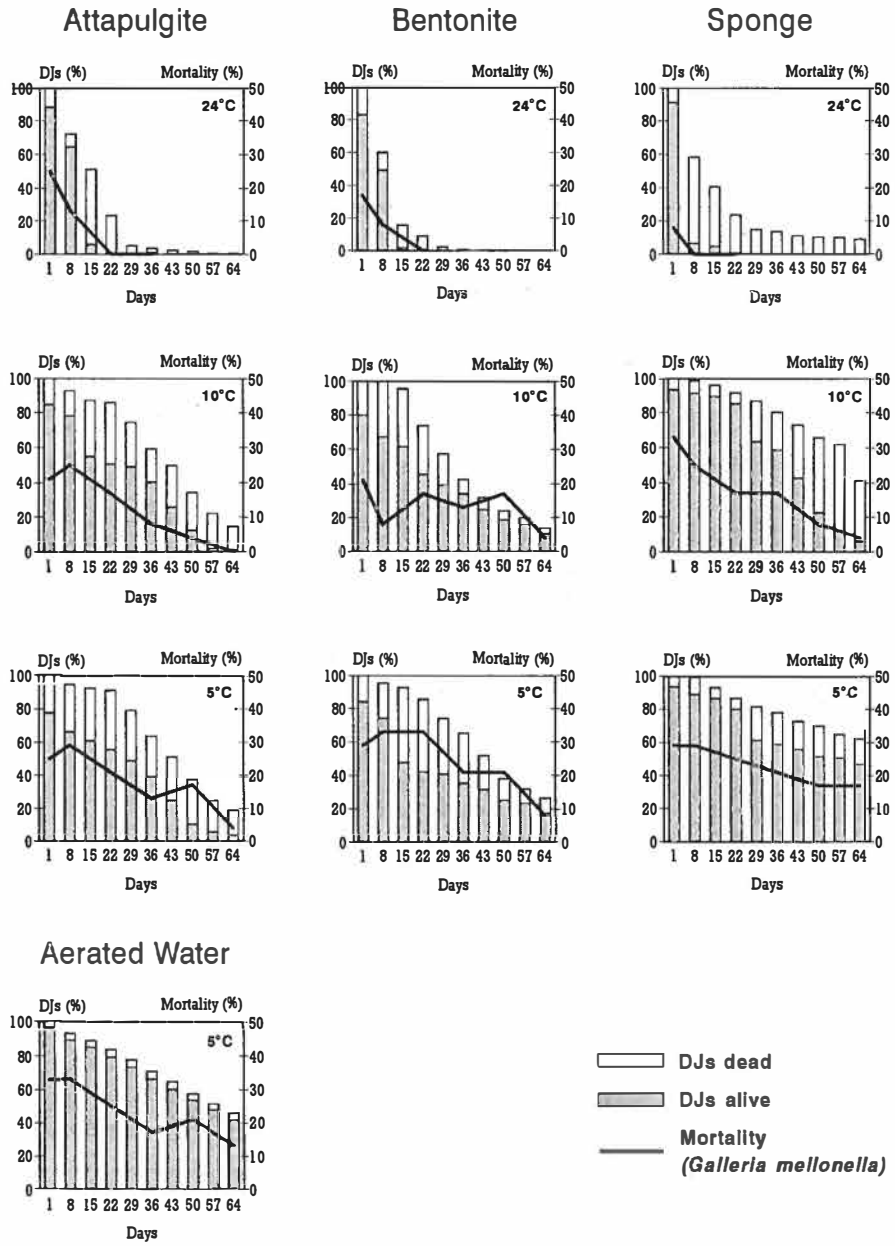


Fig. 1: Living and dead dauer juveniles (% of initial concentration) and infectivity towards *Galleria mellonella* (one nematode to one insect) of liquid culture produced *Heterorhabditis bacteriophora* formulated in attapulgite, bentonite or sponge and stored at temperatures of 24°, 10° and 5°C. Controls were kept at 0.5 Mio. DJs/ml in aerated water (Exp. 1).

In attapulgite 5%, in bentonite less than 20% and in sponge and aerated water about 40% of the DJs had survived until the end of the experiment. The final infectivity of DJs stored in sponge and water was also higher than of nematodes extracted from the clays. In aerated water the amount of dead DJs was continously on a lower level.

The experiment was conducted a second time with another batch of DJs. The results obtained matched well with those recorded here: The same effects on the survival and infectivity were obtained by the variation of media and temperature. However, in contrast to the experiment recorded here, the initial infectivity of the DJs was higher, but decreased much quicker. 36 days after formulation the infectivity and also the survival rate was already below those values recorded for the first batch.

DISCUSSION

A comparison between the different formulation media reveals that nematodes survive better when stored at low temperature in aerated water or sponge than when formulated in clays, which might be due to an overall better oxygen supply in water and sponge. If nematodes are stored at 24°C, a temperature commonly recorded during summer in temperate climates, the DJ survival is severely effected. Due to a rapid decline in the moisture content and the high migration activity of the DJs they die within two weeks after formulation. These effects were not observed when the DJs were refrigerated. They are less active and consequently can for a longer periode preserve their energy reserves.

In the second experiment the amount of living individuals decreases more rapidly compared with the results obtained in the first tests, which emphasizes the import influence of the biochemical-physiological fitness of the different DJ batches on the success of a formulation and subsequent storage. The reason for the lower survival potential of the second batch is unknown, however, it might be suggested that a lower lipid content of the DJs could have been the reason for the overall reduced survival. If this observation is always correlated with a higher initial infectivity cannot be concluded yet and needs further investigations.

The suggestion that *H. bacteriophora*, orginating from warmer climatic regions, would preferably be stored at 10° rather than at 5°C, could not be proven. On the contrary, after longer periodes of storage more DJs survived at the lower temperature. Whether even lower temperatures could be applied during storage in order to better conserve nematode infectivity and viability should be the objectiveof future experiments.

The results show that none of the tested formulation techniques gave satisfactory survival and nematode quality (infectivity) could not be preserved for a periode longer than 3 month. Consequently, the development of improved formulation techniques needs further attention in order to commercialize *H.bacteriophora* on a large scale. Objective will be the development of formualtion techniques which can guarantee a long term survival of at least 80% of the initially formulated material

and an overall high infectivity. The lower the DJ mortality the lower is the risk of a decreasing oxygen supply due to microbial decomposition of the dead nematodes. This objective can only be achieved by reducing the DJs'metabolism either by storage at lower temperatures or by means of inducing any kind of anabiotic state. However, care must be taken that nematodes can recover immediatley from the quiescent state in order to guarantee an active movement into the soil or towards the target insect after application. At the present time, the development of improved formulation techniques is the key to any kind of future large scale commercialisation of nematode biocontrol products.

REFERENCE

Ehlers, R.-U. (1995). Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. Biocontrol Sci. & Technol. **5**, in press.

ACKNOWLEDGEMENT

The provision of the nematode material by K.-H. Osterfeld, Ecogen Bio Germany GmbH, is greatfully acknowledged.

4. Entomopathogenic Fungi

MICROBIAL CONTROL OF GREENHOUSE PESTS USING ENTOMOPATHOGENIC FUNGI IN THE USA

R. Lindquist

Department of Entomology, The Ohio State University/OARDC, 1680 Madison Ave., Wooster, OH 44691, USA

The vast majority of greenhouses in the USA produce ornamental plants, not vegetables (With the exception of vegetables produced for transplanting.). In 1994, the total greenhouse area devoted to greenhouse vegetables was nearly 240 ha, while floriculture crop production area was 4300 ha. Of the floriculture crop area, only 700 ha were covered by traditional glass structures. There also are 11,000 ha of outdoor production. The wholesale value of floriculture crops in 1994 was $3.2 billion (=milliard) (USDA, 1995). The leading floriculture crop-producing states are California, Florida, Michigan, Texas and Ohio (Gray, 1995). Although the largest greenhouse company in the USA produces fresh cut flowers, most of the other large growers produce pot plants and bedding plants. There are very few monoculture greenhouse operations in the USA. Most produce a variety of crops during the year. The wholesale values of floriculture crop groups in the USA, by percentage of crop type are: bedding and garden plants (43%), potted flowering plants (22%), foliage plants (16%), cut flowers (15%), and cut greens (4%) (USDA, 1995).

Insect and mite pests can be severe pests on many of the above crop groups. The most important pests at this time are *Frankliniella occidentalis* Pergande (western flower thrips), *Aphis gossypii* Glover (melon aphid), *Myzus persicae* Sulzer (green peach aphid), *Tetranychus urticae* Koch (two-spotted spider mite), *Trialeurodes vaporariorum* (Westwood) (greenhouse whitefly) and *Bemisia argentifolii* Bellows & Perring (=*B. tabaci*, strain B) (silverleaf whitefly). Current management practices for these pests generally involve the application of pesticides. Pesticide resistance is a problem with all of the above pest groups. Growers often respond to this by making more frequent applications at higher concentrations.

However, things are changing. A recent revision of Worker Protection Standards regulations was broadened to include greenhouse workers. As a part of these regulations, pesticide labels were changed to include a time interval between application and worker re-entry into treated areas. The minimum interval is 4 hours and the maximum is 72 hours. The intervals are generally based on pesticide toxicity. Although there has been a steady move toward registration and use of less toxic, "soft" or "green", pesticide products during the past decade, even before the new regulation were implemented, the fact that many of these newer products have shorter re-entry intervals than most conventional pesticides has stimulated more development by pesticide companies and use by growers. At this time there are several "broad spectrum" insect growth regulators and plant extract products registered and used by growers. Examples include azadirachtin, cyromazine, fenoxycarb and kinoprene. Even the newest conventional pesticides, imidacloprid and pyridaben, have low enough toxicity to allow a 12 hour re-entry interval.

In addition to less toxic pesticide products, there is increasing interest in the use of introduced parasitiods and predators for insect and mite control on floriculture crops, as has been done on vegetables in northern and eastern Europe for many years. The availability of biological controls such as *Encarsia formosa* Gahan, *Phytoseiulus persimilis* Athias-Henriot, *Orius insidiosus* (Say), etc. is good (although quite expensive, because many must be shipped from Europe). Crops such as poinsettia, rose, chrysanthemum and several foliage plant species have been produced under biological control programs in some areas, but results have not been consistent. One of the problems with floriculture crops is that there are so many species and they often are attacked by multiple pests, which complicates introduction of biological controls and adds to crop production costs. A further problem with ornamental plants is a low aesthetic injury threshold. Introduced parasitoids and predators, even when successful at "controlling" a pest, may not meet requirements for pest-free plants. The use of Entomopathogenic fungi may help solve this problem in the future (Osborne and Landa, 1992).

The knowledge that insects and mites are attacked by naturally-occurring fungi is not new, but there has not been much movement toward commercial development in the USA until recently. There are several reasons for this, but all involve the ability of private companies to develop and market proprietary products. Probably, the primary stimulus for commercial development of fungi in the was caused by the extremely heavy whitefly infestations that occurred, mostly on outdoor vegetables and cotton, in California, Arizona, Florida and Texas during the past few years. For example, whiteflies caused an average of $320 million loss for the years 1991 to 1993, with a loss of 5,000 jobs in California's imperial Valley (DeQuattro, 1995). Conventional pesticide applications were not very successful for a number of reasons. Trials with fungi were quite successful. The potential of being able to provide patented products for use on thousands of hectares became very interesting to some pesticide companies. Other factors in the commercial development of fungi include advances in production and formulation technology (Brownbridge *et al.*, 1994). The companies developing the entomopathogenic fungi are quite small by corporate standards. However, if fungi are successful on major crops, we can expect that the smaller companies will become parts of larger ones.

Much of the current research and development of entomopathogenic fungi for use in greenhouses is being conducted at the University of Florida (Osborne *et al.*), the University of Vermont (Brownbridge *et al.*) and commercial companies. In addition, Sosnowska conducted experiments with PFR-97 at The Ohio State University from 1993 to 1994 while a visiting scientist from the Institute of Plant Protection in Poznan, Poland. The fungi registered or under development in the USA and their primary target pests in greenhouses are listed in the following table.

Table 1. Entomopathogenic fungi under commercial development in the USA[1]

Fungus	Potential Target Pests[2]
Beauvaria bassiana	SLWF, GHWF, WFT, GPA, TSSM
Paecilomyces fumosoroseus	SLWF, GHWF, WFT, TSSM
Metarhizium anisopliae	WFT, GPA

[1]Table modified from Brownbridge (1995).
[2]SLWF=*B. argentifolii*; GHWF=*T. vaporariorum*; WFT=*F. occidentalis*; GPA=*M. persicae* (other aphids as well); TSSM=*T. urticae*.

At this time there is only one product, Naturalis-O (Troy Biosciences), based on *Beauvaria bassiana* (Balsamo) Vuillemin, registered for greenhouse use. However, another *B. bassiana* product, Mycotrol (Mycotech Corp), is registered for use on other crops. PFR-97 (Grace Biopesticides), a product containing *Paecilomyces fumosoroseus* (Wize) Brown and Smith is in the registration process, with label approval expected shortly. PFR-97 was isolated in Florida from mealybugs. The University of Florida obtained a patent on this strain and licensed it to W.R. Grace. A third product, Back-off (Ecoscience), containing *Metarhizium anisopliae* (Metschinkoff) Sorokin, is also under development. *Verticillium lecanii*- (Zimmerman) Viégas based products are not registered in the USA, although there has been experimental evaluation (Vehrs and Parrella, 1991).

The basic approach to using fungi in greenhouses has changed. At first, fungi were applied in the expectation that the pathogen would become epidemic. The present approach is to use entomopathogenic fungi as biopesticides, making applications at regular intervals over a period of time. This is similar to the way that parasitoids and predators are used (conventional pesticides as well) to control some pests.

The following is a summary of recent research results against major pests in greenhouse experiments, based on written reports and personal communications. The most successful control using the fungi in Table 1 has been against whiteflies. Both *B. bassiana* and *P. fumosoroseus* have provided good to excellent control of *T. vaporariorum* and *B. argentifolii* (Osborne and Landa, 1992). Oil added to *P. fumosoroseus* improved control of *B. argentifolii* more than Tween 80 and a nonionic wetter (Brownbridge *et al.*, 1995).Thrips (*F. occidentalis*) control has been good with PFR-97, particularly when applications were made to both plants and potting mix surfaces (Sosnowska and Lindquist, unpublished). Drenches of suspensions of all three fungi listed in Table 1 significantly reduced *F. occidentalis* adult emergence (Anonymous, 1995). Azadirachtin combined with PFR-97 increased control of *F. occidentalis*, compared with either product used alone (Sosnowska and Lindquist, 1994). Control of aphids has been mixed. Sosnowska's experiments with PFR-97 against *A. gossypii* in greenhouse experiments were not successful. Brownbridge reported that strains of *B. bassiana* and *M. anisopliae* caused 100% mortality of *M. persicae* within 4 days.

Fungi can be applied as high volume sprays, low volume sprays, soil applications of dry formulations and drenches of spore suspensions. High volume sprays are used most often, but other methods are promising. Osborne (pers. comm.) applied PFR-97 to a greenhouse poinsettia crop using an electrostatic sprayer, with excellent results against *B. argentifolii*.. More experiments with low volume applications are underway in Vermont (Brownbridge, pers. com.) and Ohio.

Will fungi be accepted by growers in the USA? As with anything new and different there will necessarily be an adaptation and education process. A few growers will accept the fungal products readily, because they will know how to utilize them in pest management programs. Most will take a "wait and see" attitude, using the experiences of other leading growers as well as new research and extension information to slowly integrate fungal formulations into their pest management programs. Researchers and crop advisors will need to educate themselves and growers on proper application dosages and intervals.

LITERATURE CITED

ANONYMOUS. 1995. Insect killing fungi as a weapon in greenhouse pest management. American Floral Endowment Update, June, 1995: 2.

BROWNBRIDGE, M. 1995. Good fungi for your crop protection program. Presentation at Ohio International Floral Short Course, Cincinnati, OH. 5pp.

BROWNBRIDGE, M., MCLEAN, D.L., PARKER, B.L. AND SKINNER, M. 1994. Use of fungal pathogens for insect control in greenhouses. Proceedings, 10th Conf. on Insect & Disease Management on Ornamentals. K. Robb, ed. pp 7-20.

DEQUATTRO, J. 1995. Whitefly fungus on way to growers. Agricultural Research/May 1995: 16-17.

GRAY, H.E. 1995. Greenhouse construction: on solid ground. Greenhouse Grower 13(6): 48-50.

OSBORNE, L.S., LANDA, Z. 1992. Biological control of whiteflies with entomopathogenic fungi. Fl. Entomologist 75(4): 456-471.

SOSNOWSKA, D., AND LINDQUIST, R. 1994. Wykorzystanie owadobójczego gryzyba Paecilomyces fumoso-roseus (Wize) I preparatu Margosan* w biologicznym zwalczanui wciornastka zachoniego (Frankliniella occidentalis, Pergande). Ochrona Roslin 2-3.

USDA. 1995. Floriculture crops: 1994 summary. National Agr. Statistical Service Sp Cr 6-1(95).

VEHR, S.L., and PARRELLA, M.P. 1991. Aphid problems increase on Ornamentals. California Agriculture 45(1): 28-29.

NATURAL OCCURRENCE OF ENTOMOPHTHORALES ON CEREAL APHIDS: A COMPARISON OF PREVALENCE STUDIES AND CADAVERS COUNTS

Jørgen Eilenberg, Tove Steenberg & Charlotte Nielsen

Department of Ecology and Molecular Biology
Royal Veterinary and Agricultural University
Bülowsvej 13
DK 1870 Frb. C., DENMARK

Summary

In 1994, cereal aphids (*Sitobion avenae, Rhopalosiphum padi* and *Metopolophium dirhodum*) were studied with respect to occurrence of entomopathogenic fungi from Entomophthorales (*Entomophthora planchoniana, Erynia neoaphidis, Conidiobolus obscurus*). Two methods were used for assessing the occurrence of fungal pathogens in the population: Sampling and incubation of living individuals and sampling of dead, fungus killed aphids (cadavers). No correlation was found between the two expressions of the natural occurrence of entomopathogenic fungi, the former should, however, be regarded as the correct expression of the prevalence of fungi in an aphid population.

Introduction

It is well-known, that a number of entomopathogenic fungi from Entomophthorales may infect cereal aphids (*Sitobion avenae, Rhopalosiphum padi* and *Metopolophium dirhodum*) of economic importance in Europe (eg. Robert *et al.*, 1973; Wilding & Perry, 1980; Keller & Suter, 1980). The most important species from Entomophthorales infecting cereal aphids include *Entomophthora planchoniana, Erynia neoaphidis, Conidiobolus obscurus* and *Neozygites fresenii*.

Studies documenting the occurrence of entomopathogenic fungi in insect populations at a quantitative level are desirable as a basis for understanding the population dynamics and as a prerequisite for future biological control. The most used term for infection level is "prevalence" defined as the number of hosts afflicted with that disease at a given point of time (Fuxa & Tanada, 1987). In order to get information about prevalence, a frequent method is sampling and incubating living individuals, allowing infections to develop the typical macro- and microscopic features (Fuxa & Tanada, 1987). Several studies on entomopathogenic fungi on cereal aphids are based on calculation of prevalence (Dean & Wilding, 1973; Keller & Suter, 1980; Feng *et al.*, 1991).

Quantitative sampling of dead, fungus killed insects will in many cases be useless due to difficulties to find the quickly disintegrating cadavers for example in the soil. In the particular case of aphids in cereals, specimens killed by either a parasitoid or a fungus will stay fixed to the leaves as cadavers and are, before they fall off or are eaten, easy to find. Quantitative studies on the occurrence of parasitoids in such aphid populations has therefore often used counts of numbers of cadavers as an expression of the population density of the parasitoid (Feng *et al.*, 1991). Counting of cadavers has also been used as an estimation of the infection level of entompathogenic fungi (Dedryver, 1978; Steinkraus *et al.*, 1995).

The purpose of our study in 1994 was to compare the two set of methods for estimation of fungal infection in a population of cereal aphids: sampling and incubation of living individuals and counting of cadavers.

Materials & methods

The study area was a 3.8 ha winter wheat field (Aagerup, Zeeland, Denmark) without pesticide treatment.

Aphid density: 5-20 tillers were collected on each of 32 sampling plots, evenly distributed throughout the field. Aphids were carefully removed and determined to species level and instar. The number of living aphids per cut tiller was used as an expression of the aphid density.

Cadaver counts: Fungus killed cereal aphids were quantified from 10-20 tillers. If possible, the aphids and fungi were determined to species level.

Prevalence: Living aphids were carefully removed from the tillers and individually tranferred for incubation in plastic cups (25 ml) with 2% water-agar in the bottom and a plastic lid. Between 24 and more than 1300 aphids were incubated per week. Dead aphids were removed daily for a period of 7 days and were incubated in moist chambers. Both aphid and fungus were determined to species and instar level.

Results

In the period June 6 to June 27, the population of cereal aphids developed rapidly, whereafter it quickly decreased (fig. 1). The predominant aphid species in 1994 was *R. padi*, accounting for more than 90 % of the total population in the crop.

The cadaver counts proved fungal diseases to be prevalent from the beginning of June and onwards with a distinct maximum in mid July. About 55 % of the sampled aphids on July 18 were fungus-killed (cadavers). In most cases it was not possible to determine neither the aphid species nor the fungus species due to a lack of characters caused by for example overgrowing by saprophytic fungi.

The prevalence as calculated by sampling and incubation of living individuals revealed a maximum in late June (about 30 % infection), but with no distinct peak. The predominant fungus was *E. planchoniana*, which was responsible for the high infection levels.

Discussion

The two methods for estimation of infection levels gave a rather different picture of the dynamics and of the time and magnitude of the maximum. Obviously, they can not be compared directly.

The main advantages of sampling and incubation of living individuals are:

- Determination to species level of both aphid and fungus is possible
- An exact and actual picture of the infection level as prevalence is obtained

The disadvantages are:

- The method is time consuming
- The data are left with the problem of interpreting, whether the infection revealed in the laboratory would also develop in the field

The main advantages of cadaver counts are:

- The method will quickly give data about density of true fungus killed aphids
- The method, when including counts of spore discharge from cadavers, will give a good estimate of the spore density in the crop
- The method is easy to combine with studies on parasitoid populations

The main disadvantages are:

- In system with more than one significant aphid host and more than one significant fungal pathogen the method will not offer detailed information about either of these
- The total number of cadavers does not give an actual picture of infection level, since cadavers are accumulated over time on the leaves. Further, this accumulation can be regarded as weather dependent: Heavy rain will wash off cadavers. In 1994, which had a long period of dry weather, the accumulation of cadavers was probably enhanced

The balance between advantages and disadvantages of the two methods and the choice of study to be performed depends on the system to be studied and the precision to be obtained. We will especially point out that the studied system with several aphid hosts and several fungal pathogens can not be studied without including prevalence studies. Other system with one aphid species and only one predominant fungus may be more appropriate to study only with cadaver counts (Steinkraus *et al.*, 1995).

Finally we will, however, also point out that terms like prevalence and incidence are defined properly within insect epizootiology (Fuxa & Tanada, 1987). They should therefore be given priority for studies leading to easier and more direct comparison with other studies on ecology of insect pathogens.

References

DEAN G.J.W. & WILDING N., 1973. Infection of cereal aphids by the fungus *Entomophthora*. Ann. Appl. Biol. 73:133-138.

DEDRYVER, C.A., 1978. Facteurs de limitation des populations d'*Aphids fabae* dans l'ouest de la France. III Répartition et incidence des différentes espeèce d'*Entomophthora* dans les populations. Entomophaga. 23:137-151.

FENG, M.G.; JOHNSON, J.B. & HALBERT, S.E. 1991. Natural Control of Cereal Aphids (Homoptera: Aphididae) by Entomopathogenic Fungi (Zygomycetes: Entomophthorales) and Parasitoids (Hymenoptera: Braconidae and Encyrtidae) on Irrigated Spring Wheat in Southwestern Idaho. Environmental Entomology. 20:1699-1710.

FUXA, J.R. & TANADA, Y. 1987. Epidemiological concepts applied to insect epizootiology. In Epizootiology of Insect Diseases. Fuxa, J.R. & Tanada, Y. (eds.). John Wiley & Sons, New York. pp.4-41.

KELLER, S. & SUTER, H. 1980. Epizootiologische Untersuchungen über das *Entomophthora* - Auftreten bei feldbaulich wichtigen Blattlausarten. Acta Ecologica. 1:63-81.

ROBERT, Y.; RABASSE, J.M. & SCHELTES, P. 1973. Facteurs de limitation des populations d'*Aphis fabae* Scop. Dans l'ouest de la France. Entomophaga. 18:61-75.

STEINKRAUS, D.C.; HOLLINGWORTH, R.G. & SLAYMAKER, 1995. Prevalence of *Neozygites fresenii* (Entomophthorales: Neozygitaceae) on Cotton Aphids (Homoptera: Aphididae) in Arkansas Cotton. Environmental Entomology. 24: 465-474.

WILDING, N. & PERRY, J.N. 1980. Studies on *Entomophthora* in populations of *Aphis fabae* on field beans. Ann. Appl. Biol. 94:367-378.

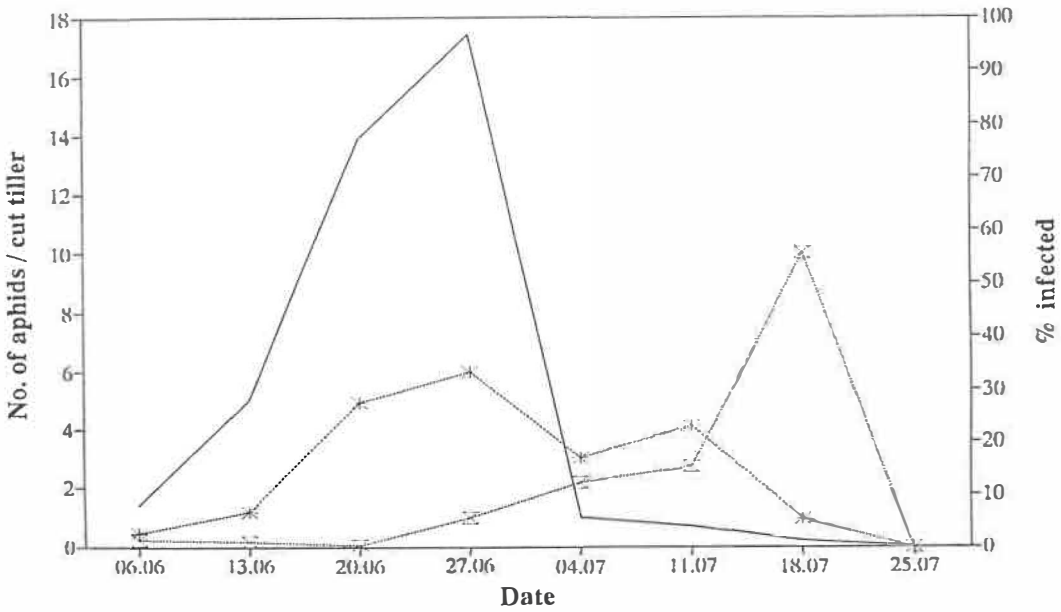


Fig. 1

Population development of cereal aphids (*Rhopalosiphum padi, Sitobion avenae* and *Metopolopium dirhodum*) and occurrence of entomopathogenic fungi assessed by prevalence and cadaver counts.

——— = Cereal aphid population (no living aphids per cut tiller)

---*--- = Prevalence of entomopathogenic fungi (% infection in living, incubated individuals)

---□--- = Infection level of entomopathogenic fungi measured as percentage fungus killed aphids (cadavers) (the percentage is measured of the total of living aphids and fungus-killed aphids)

THE EFFECT OF SOIL-BORNE ENTOMOGENOUS FUNGI ON THE MYCOSES OF THE COLORADO POTATO BEETLE DURING HIBERNATION PERIOD.

Ryszard Miętkiewski, Anna Sapieha, Cezary Tkaczuk

Agricultural and Pedagogical University, ul. Prusa 14, 08-110 Siedlce, Poland.

Introduction

Little is known about the influence of kind of soil on the mycoses of L. decemlineata. Generally the kind of soil has a big influence on the spectrum of entomopathogenic fungi (Vanninen et al. 1989, Kleespies et al. 1989, Miętkiewski et al. 1991, Miętkiewski et al. 1991/1992.

The objective of this present study was to determine the fungi which reduce the Colorado potato beetle (CPB) population in Poland during hibernation period. In addition the spectrum of entomopathogenic fungi in four kinds of soil was established by means of "the insect bait method" and in these soils CPB mycoses during hibernation were determined.

Materials and methods

Dead beetles of the L. decemlineata delivered in early spring by twenty Quarantine and Plant Protection Stations covering different regions of Poland were used in order to determine the natural spectrum of entomopathogenic fungi reduceing this pest during hibernation.

In the pot experiment at the beginning of September 20 beetles were put in each 5 litre pot and next those pots were buried in soil. 5 pots were allowed for each combination of soil (20 pots in all). In spring a definite relationship was established beetwen the mortality of the beetle and the presence of entomopathogenic fungi, which had been isolated from dead specimens of the beetle.

Four kinds of soil were used in the experiment. There were two types of soil taken from potato plantations, one sample from forest soil and one from meadow soil.

In another experiment the spectrum of entomopathogenic fungi in each kind of soil was established by using the insect bait method. Soil was placed in plastic Petri dishes and 10 Galleria mellonella larvae were added to each of the 10 Petri dishes per treatment (100 larvae in all). Petri dishes with soil and larvae were incubated at 18°C and 28°C and larval mortality was monitored after 7 days and then every 4 days.

Results

The spectrum of entomopathogenic fungi received from dead beetles was homogenous in the whole area of Poland. B. bassiana was the dominant species infecting average 21 % of specimens. P. farinosus was isolated from 10 % of dead beetles. Verticillium lecanii appeared only sporadicly. (Tab. 1).

Table 1. Entomopathogenic fungi isolated from dead Colorado potato beetles

Species of fungus	Year of observation					
	1987		1990		1991	
	a	b	a	b	a	b
Beauveria bassiana	44	21,1	82	24,4	28	16,2
Paecilomyces farinosus	21	10,1	39	11,6	15	8,7
Verticillium lecanii	12	5,7	1	0,3	2	1,2

Explanations:
a - number of investigated beetles
b - percentage of specimens with symptoms of mycoses

In the pot experiment the highest mortality of the beetle during hibernation was noticed in potato derived soils. B. bassiana was the dominant species isolated from dead beetles. In the soil from field I this fungus killed 35 % of beetles, and in the soil from field II - 42 %.

Paecilomyces farinosus was the second most common species with regard to fungal occurrence on the hibernating beetle. This species colonized the highest number of beetles in the soil from potato field I.

Only one beetle was infected by M. anisopliae. It was in the meadow soil. The highest number of living beetles after hibernation were found in forest soil. (Tab. 2).

Differences between entomopathogenic fungi in different kinds of soil obtained from G.mellonella larvae and from the beetle L. decemlineata were observed. B. bassiana appeared most frequently on G. mellonella larvae in forest soil. In arable soil it did not appear.

M. anisopliae was noted sporadicly on the beetle of L. decemlineata but in the insect bait experiment it was observed in all types of soil. In the meadow soil 38 % of G. mellonella larvae were infected at 18°C and 73 % - at 28°C. In another soils the same fungus infected G. mellonella larvae only at the temperature of 28°C.

P. fumosoroseus was not found on L. decemlineata but was observed on the insects bait in all kinds of soil at 18°C.

On the dead G. mellonella larvae P. farinosus appeared sporadicly but was often isolated from L. decemlineata beetles.

In the soil from potato field II M. anisopliae was isolated only from G. mellonella larvae.

Table 2. Mortality (%) of Leptinotarsa decemlineata adults and Galleria mellonella larvae caused by entomopathogenic fungi in different kinds of soil.

Kinds of soil	Species of insect	Temp. (°C)	Species of fungus				
			B.b	M.a	M.f	P.fa.	P.fu.
Meadow soil	L.d.	X	-	1,0	-	-	-
	G.m	18	-	38,0	1,0	-	7,0
		28	-	73,0	3,0	-	-
Forest soil	L.d.	X	20,0	-	-	6,0	-
	G.m.	18	3,0	-	-	3,0	11,0
		28	2,0	8,0	-	-	-
Soil from potato plantation Field I	L.d.	X	35,0	-	-	10,0	-
	G.m.	18	8,0	-	-	-	69,0
		28	-	83,0	-	-	-
Soil from potato plantation Field II	L.d.	X	42,0	-	-	5,0	-
	G.m.	18	3,0	3,0	-	2,0	70,0
		28	63,0	63,0	2,0	-	-

Explanations:

B.b. - Beauveria bassiana, M.a. - Metarhizium anisopliae,
M.f. - Metarhizium flavoviridae, P.fa. - Paecilomyces farinosus
P.fu. - Paecilomeces fumosoroseus
L.d. - Leptinotarsa decemlineata
G.m. - Galleria mellonella
X - temperature of soil during hibernation period of L.decemlineata

Disscusion

The spectrum of entomopathogenic fungi established by means of G. mellonella larvae was different from that collected from dead Colorado potato beetles in the same soil.

M. anisopliae was the dominant species isolated on G. mellonella larvae from meadow soil at 18°C and 28°C, and from arable soils at 28°C. P. fumosoroseus was the dominant species isolated

from arable soil at 18°C. B. bassiana was the dominant species in forest soil at 18°C but in other kinds of soil appeared very rarely.

This data is similar to results obtained by Miętkiewski et al. (1991), Kleespies et al. (1989), Miętkiewski and Miętkiewska (1993).

The differences between the spectrum of entomopathogenic fungi found on dead CPB and on insects bait may be influenced by many factors.

In the case of M. anisopliae, which was isolated only from one adult specimen of the L. decemlineata beetle, the limiting factor was temperature during hibernation period. This termophilic species infected G. mellonella mainly at 28°C. Similar results were obtained by Miętkiewski et al (1994).

The higher mortality of CPB in arable soils caused by B. bassiana and P. farinosus may be explained by the susceptibility of this host to infection by both fungi. This fact may also be explained by the way in which both B. bassiana and P. farinosus strains taken from potato field soils have higher patogenicity to CPB than strains from other kinds of soil which have not had contact with the beetle. This point of view confirms Bajan et al. (1981) who found that the highest mortality of the pest is caused by strains of fungi derived from the same area as the infested by pest.

References

1. Bajan C., Fedorko A., Kmitowa K. 1981. Wpływ wybranych czynników na efektywność mikroorganizmów regulujących liczebność stonki ziemniaczanej. Mat. Sesji Nauk. IOR 21: 259-266.
2. Kleespies R., Bathon H., Zimmermann G. 1989. Untersuchungen zum naturlichen Vorkommen von entomopathogenen Pilzen und Nematoden in verschidenen Boden in der Umgebung von Darmstadt. Gesunde Pflanzen. 41 (10): 350-355.
3. Miętkiewski R., Miętkiewska Z. 1993. Grzyby entomopatogeniczne w glebie. Acta Mycol. 28 (1): 77-82.
4. Miętkiewski R., Tkaczuk C., Zasada L. 1991/1992. Występowanie grzybów entomopatogennych w glebie ornej i łąkowej. Acta Mycol. 27 (2): 197-203.
5. Miętkiewski R., Tkaczuk C., Zurek M., Van der Geest L.P.S. 1994. Temperature requirements of four entomopathogenic fungi. Acta Mycol. 29 (1): 109-120.
6. Miętkiewski R., Zurek M., Tkaczuk C., Bałazy S. 1991. Występowanie entomopatogennych grzybów w glebie ornej, leśnej oraz ściółce. Roczn. Nauk Roln. E 21 (1/2): 61-68.
7. Vanninen I., Husberg G.B., Hokkanen H.M.T. 1989. Occurrence of entomopathogenic fungi and entomoparasitic nematodes in cultivated soils in Finland. Acta Entomol. Fennica 53:65-71.

NATURAL OCCURRENCE OF ENTOMOPATHOGENIC FUNGI ON THRIPS (THYSANOPTERA) IN A DANISH WHEAT FIELD

CHARLOTTE NIELSEN, TOVE URUP MADSEN, JØRGEN EILENBERG AND HOLGER PHILIPSEN

Department of Ecology and Molecular Biology, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, DENMARK

Summary

An investigation of the populations of thrips in a Danish winter wheat field was carried out with the focus on the natural occurrence of insect pathogenic fungi. The most frequently encountered species of thrips throughout the season was *Limothrips cerealium* Haliday (Thysanoptera: Thripidae), which accounted for about 80% of the adult thrips population. The presence of *Verticillium lecanii* (Zimmermann) Viégas, *Beauveria bassiana* (Balsamo) Vuillemin and *Neozygites parvispora* (MacLeod *et al.*) Remaudière & Keller was documented.

Introduction

Limothrips cerealium Haliday and *Limothrips denticornis* Haliday (Thysanoptera: Thripidae) are occasional pests of cereals in Northern Europe. Feeding by *Limothrips* spp. on leaves and especially flag leaves results in loss of pigmentation which causes yield reduction. In the ear it may reduce seed germination (Oakley, 1980; Chisholm & Lewis, 1984).

Carl (1975) and MacLeod *et al.*(1976) have reported the fungus *Neozygites parvispora* (MacLeod *et al.*) Remaudière & Keller as a pathogen frequently causing epizootics in populations of *Thrips tabaci* Lind. on onion crops in Switzerland. Furthermore Samson *et al.* (1979) have observed *Entomophthora thripidum* Samson, Ramarkers *et* Osvald as a fungal pathogen causing epizootics in *T. tabaci* populations in glasshouses in the Netherlands. Qualitative data obtained in thrips populations in cereals in Denmark showed that *N. parvispora* apparently caused epizootics in 1990 and 1993, and *V. lecanii* was found at enzootic level (Steenberg, Philipsen & Eilenberg, unpubl.).

The aim of this investigation was to study the population of thrips focusing on the natural occurrence of insect pathogenic fungi. Thrips were studied during the 1994 season in one winter wheat field in Denmark.

Materials and methods

One 13 ha winter wheat field situated at Ågerup (Zealand, Denmark) was selected for the study. Samplings for thrips were performed in the 3,8 ha south west end of the field. The sampling area had not been subjected to pesticide treatments and was bordered on one side by a hedge. Samplings took place weekly from the end of April 1994 until the beginning of August 1994. Plant growth stage (Zadoks scale) was determined in the field at each sampling date. Weather data (temperature, relative humidity and precipitation) were obtained from a weather station 10 km away.

Population of thrips

The sampling area was divided into nine plots. Within each of these plots a small square (1,5 x 1,5 m) was chosen for sampling of wheat tillers. Ten tillers were chosen at random from each square at each sampling date. Tillers were cut below the two uppermost leaf sheathes, and after earing each sample was divided into two, one consisting of ears and one consisting of the lower part of the plants (stem, leaf sheathes, and leaves). The ear fractions were immediately placed in an extraction bottle in the field and thrips were driven out from the ears with turpentine (Lewis, 1960). The lower part of the plants were transported to the laboratory where thrips were removed carefully with a brush and transferred to 70% ethanol. Both adult thrips and thrips larvae were counted, and adult thrips were identified under the microscope (Maltbæk, 1932; Palmer *et al.*, 1992 and Moritz, 1994). Three yellow sticky traps were placed in the field to detect flight activity of the thrips during the season.

Natural occurrence of entomopathogenic fungi on thrips

This study was based mainly on samples collected by sweeping net. It was attempted to sweep 150 adult thrips consisting of at least 100 *Limothrips cerealium*. Immediately after catching, the thrips were transferred ten by ten to glass petri-dishes (9 cm) which contained moistered filter paper in the bottom. Condensation of water was avoided by covering the dishes with semi-permeable pvc cling film. Food (wheat leaves) was provided directly on the filter paper. Incubation took place in the laboratory at 20°C and mortality was recorded daily for six days. Dead thrips were transferred to 25 ml plastic cups with 5 ml 2% water-agar to facilitate sporulation of possible entomopathogenic fungi. Entomopathogenic fungi were identified under the microscope using the morphology of the conidiophores and the spores as key characters (Carl, 1975; MacLeod *et al.*, 1976; Brady,1979a+b).

Results

Population of thrips

Results of the investigation of the population of thrips in a winter wheat field are shown in table 1. Seven species occurred on winter wheat. *L. cerealium*, *L. denticornis* and *Thrips* spp. (probably *Thrips angusticeps* Uzel.) were the most common species throughout the season. *L. cerealium* thus accounted for 83,4% of the total sampled thrips population (700 adult individuals).

L. cerealium and *Thrips* spp. mostly fead on ears whereas *L. denticornis* mostly fead on the lower part of the plants.

Table 1: Composition among sampled thrips species (700 adult individuals) from cut winter wheat tillers between April and August 1994 at Ågerup, Denmark .

Species	English name	Percentage of total
Aptinothrips stylifer	Grass thrips	0,1%
Frankliniella tenuicornis		0,7%
Haplothrips aculeatus		0,1%
Limothrips cerealium	Grain thrips	83,4%
Limothrips denticornis	Barley thrips	5,4%
Stenothrips graminum	Oat thrips	0,3%
Thrips spp.		9,0%
Unidentified because of damage during handling		1,0%

Immigration of thrips began in late April (Zadoks 26-29) and continued for a couple of weeks. The first thrips larvae were found the first of June (Zadoks 37-39). The density of thrips reached a maximum of three larvae and two adults per tiller in the beginning and the end of July respectively. The most important emigration started in late July and lasted only a few days resulting in almost no thrips in the beginning of August. Considering the weather, the flight activity and observations of males June 29´th it is presumed that two generations of *L. cerealium* occurred in winter wheat in 1994.

Natural occurrence of entomopathogenic fungi on thrips

Results of the investigation of the occurrence of entomopathogenic fungi on thrips sampled in Ågerup are shown in table 2. From the Hyphomycetes *Beauveria bassiana* (Balsamo) Vuillemin and *Verticillium lecanii* (Zimmermann) Viégas were found to occur on thrips, and from the Entomophthorales *N. parvispora* was found. *V. lecanii* was the most common species in the population of thrips, and was isolated from a range of species whereas *N. parvispora* was only found on *L. denticornis*.

B. bassiana and *V. lecanii* were found on *L. cerealium* throughout the season but always in small numbers and without tendency towards epizootic development (see fig. 1). *V. lecanii* was occasionally found on still living *L. cerealium*. At one date (20/7 1994) both males and females of *L. cerealium* were collected and incubated. A χ^2-test showed that there was higher prevalence of *V. lecanii* on males than on females (significant at the 5% level). For *B. bassiana* the ratio of infected females and males was about the same.

Sampling in early spring and late autumn was carried out in litter. *V. lecanii* and *B. bassiana* were documented on these thrips.

Table 2: Naturally occurring insect pathogenic fungi on thrips collected in winter wheat Ågerup, 1994. The number in brackets indicates the number of individuals incubated throughout the season and the number of individuals found with the respective entomopathogenic fungi.

Species of thrips	*Verticillium lecanii*		*Beauveria bassiana*		*Neozygites parvispora*	
Imagines:						
Frankliniella tenuicornis (6)	(1)	16,7%				
Limothrips cerealium ♀ *(1809)*	(72)	4,0%	(34)	1,9%		
Limothrips cerealium ♂ *(277)*	(47)	17,0%	(5)	1,5%		
Limothrips denticornis ♀ *(261)*	(21)	8,0%	(4)	1,5%	(2)	0,8%
Limothrips denticornis ♂ *(61)*	(9)	14,8%	(5)	8,2%		
Stenothrips graminum (23)	(2)	8,7%				
Thrips spp. *(63)*	(2)	3,2%	(1)	1,6%		
Larvae:						
Limothrips cerealium (7)	(3)	42,9%	(2)	28,6%		
Limothrips denticornis (109)	(17)	15,9%	(6)	5,5%		

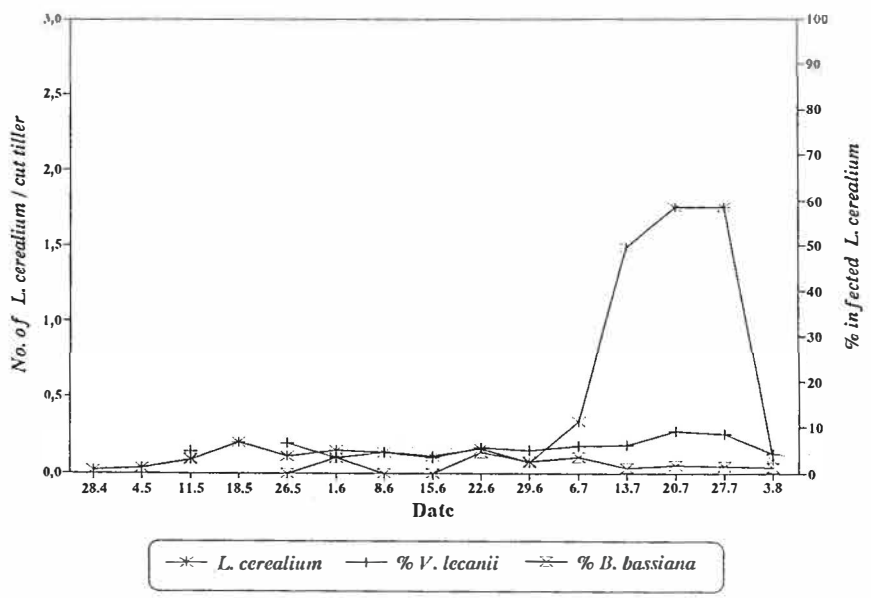


Fig. 1: Density of *Limothrips cerealium* and prevalence of fungal pathogens on *L. cerealium* in winter wheat in Ågerup 1994.

Discussion

The most frequently encountered species of thrips in winter wheat was *L. cerealium*. This result is in accordance with earlier records in other countries in Northern Europe (Johannson, 1938; Lewis, 1959; Holtmann, 1962; Franssen & Mantel, 1965; Patrzich & Klumpp, 1991).

During the 1994 season no epizootics of fungi in thrips populations at the sampling area occurred. The reasons could be both unsuitable climatic conditions and low densities of thrips. During the 1994 season the density of thrips remained low throughout the season and between 30/6 and 1/8 1994 the temperature was unusually high with no precipitation, however dew on the plants was often observed. Carl (1975) reported that epizootics in *T. tabaci* populations with *N. parvispora* allways occurres late in the season. The beginning of epizootics coincides with the onset of low night temperature frequently causing the formation of dew on plants. He suggested as an explanation that hibernating resting spores develope very slowly. Carl (1975) suggested further that epizootics late in the season is largely dependent on host density, regardless of suitable environmental conditions such as temperature and humidity.

V. lecanii and *B. bassiana* were found to infect both males, females and larvae of *L. cerealium* and *L. denticornis*. Even though the prevalence never reached more than 10%, we suggest that the the fungi may play an important role if the males and females get infected before mating or if the females get infected before the end of their oviposition.

V. lecanii and *B. bassiana* were documented on thrips in the litter, and it is hypothesized that these fungi thus may play a role as mortality factors in early spring and late autumn. *V. lecanii* was found on still living thrips and it is therefore possible that still living thrips may play a role in the transmission of the inoculum, also to other insect hosts.

Much more research on the influence of climatic and biotic factors is needed to understand the effect of infection with entomopathogenic fungi in thrips populations. This study indicates that future work should take in to account thrips density, climatic factors, indirect effect of infection with entomopathogenic fungi and transmission of inoculum with still living thrips, to understand better their effect on the population dynamics of thrips.

Acknowledgement

The present study was carried out as a part of the Center for Agricultural Biodiversity supported by The National Environmental Research Programme.

References

BRADY, B. L. K., 1979a. *Beauveria bassiana*. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 602. 2pp.

BRADY, B. L. K., 1979b. *Verticillium lecanii*. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 610. 2pp.

CARL, K. P., 1975. An *Entomophthora* sp. [Entomophthorales: *Entomophthoraceae*] pathogenic to *Thrips* spp. [*Thysan.: Thripidae*] and its potential as biological control agent in glasshouses. Entomophaga. 20(4):381-388.

CHISHOLM, I.F. & LEWIS, T., 1984. A new look at thrips (Thysanoptera) mouthparts, their action and effects of feeding on plant tissue. Bull. Ent. Res. 74:663-675.

FRANSSEN, C.J.H. & MANTEL, W.P., 1965. Tripsen in graangewassen. (Levenswijze, economische betekenis en bestrijding I. Levenswijze. Versl. landbouwk. Onderz. No. 662. 97 pp.

HOLTMANN, H., 1962. Untersuchungen zur Biologie der Getreide-Thysanopteren I. Z. Angew. Ent. 51:1-41.

JOHANNSON, E., 1938. Studier rörande de på gräs och sädesslag levande tripsarnas biologi och skadegörelse. 1. I vetefält och vallar förekommande associationsformer samt skadegörelsens natur och omfattning. Stat. Växtskyddsanst. Medd. 24:1-65.

LEWIS, T., 1959. The annual cycle of *Limothrips cerealium* Haliday (Thysanoptera) and its distribution in a wheat field. Ent. Exp. Appl. 2:187-203.

LEWIS, T., 1960. A method for collecting Thysanoptera from Gramineae. Entomologist 93:27-28.

MACLEOD, D. M.; TYRRELL, D. & CARL, K. P., 1976. *Entomophthora parvispora* sp. Nov., a pathogen of *Thrips tabaci*. Entomophaga 21(3):307-312.

MALTBÆK, J., 1932. Frynsevinger eller blærefødder (Thysanoptera). Danmarks Fauna 37. 146pp.

MORITZ, G., 1994. Pictorial key to the economically important species of Thysanoptera in Central Europe. OEPP/EPPO Bulletin 24:181-208.

OAKLEY, J.N., 1980. Damage to barley germ by *Limothrips* spp. (Thysanoptera: Thripidae). Pl. Path. 29:99.

PALMER, J.M.; MOUND, L.A. & DU HEAUME, G.J., 1992. THYSANOPTERA (2). In IIE Guides to insects importance to man.73pp. Betts, C.R. (Eds.). Antony Rowe, Chippenham.

PATRZICH, R. & KLUMPP, M., 1991. Vergleich der phytophagen und räuberischen Thripse (Thysanoptera) auf unterschiedlich bewirtschafteten Weizenfeldern in Hessen. PflKrankh. 98(5):464-470.

SAMSON, R. A.; RAMARKERS, P. M. J. & OSWALD, T., 1979. *Entomophthora thripidum*, a new fungal pathogen of *Thrips tabaci*. Canadian Journal of Botany. 57:1317-1323.

CORDYCEPIOIDEUS BISPORUS, A NATURALLY OCCURRING FUNGAL PATHOGEN ON TERMITE ALATES IN KENYA

G.S. OCHIEL[1], J. EILENBERG[2], W. GITONGA[1], J. BRESCIANI[2], & L. TOFT[3].

1.Kenya Agricultural Research Institute (KARI), National Agricultural Research Centre (NARC), Muguga, P.O. Box 30148, Nairobi, KENYA
2.Dept. of Ecology and Molecular Biology, Royal Veterinary and Agricultural University (RVAU), Bulowsvej 13, DK 1870, Frederiksberg-C, Copenhagen, DENMARK
3.Biotechnology Dept., Danish Technological Institute (DTI), Postbox 141, Gregersenvej, DK 2630, Taastrup, DENMARK

ABSTRACT:
This paper presents the results of an exploratory survey for entomopathogenic fungi on termite species in Kenya, *in vitro* isolations and culture of a termite-specific fungal pathogen on mycological media and a field sampling programme to investigate the occurrence of a termite-specific fungal pathogen. A survey carried out from 1991 to 1993, diagnosed *Cordycepioideus bisporus* Stifler, on mummified *Macrotermes subhyalinus* Rambur alates, from Isiolo (North-eastern Kenya) and from Kajiado (South-eastern Kenya) respectively. Electron and light microscopic studies of the morphology of the diagnostic structures of *C. bisporus* are ongoing. *C. bisporus*, previously documented on termite alate hosts in East Africa and Mexico, exists in perfect (teleomorph, ascospore-producing) and imperfect (anamorph, conidia-producing) forms. Three teleomorph and five anamorph isolates of *C. bisporus* were obtained from infected *M. subhyalinus* alate hosts and cultured *in vitro* on various mycological media. Sabouraud-dextrose-agar (SDA), SDA-egg-yolk and Molisch-agar media gave the best results for ascospore production and mycelial growth of a *C. bisporus* teleomorph isolate. Results of a monthly sampling programme at a selected field site in Kajiado (November 1993-April 1995), showed a correlation between the "short-rains season" (November/December) and the occurrence of *C. bisporus* anamorph on *M. subhyalinus* alates.

Key words: Entomopathogenic fungi, survey, field sampling, occurrence, *Cordycepioideus bisporus*, anamorph, teleomorph, culture media, termite alates, Kenya.

Paper presented at IUBS/IOBC-WPRS/EPS, 5th European Meeting, Microbial Control of Pests, August 27-1 September 1995, Poznan, Poland

INTRODUCTION

Information on natural occurrence of specific fungal pathogens in termite populations is scanty. A few reports on termite-specific fungi are available in the literature. The Ascomycete fungal genus *Cordycepioideus* has only two known species, *C. bisporus* Stifler, recorded on *Macrotermes natalensis* (Haviland) alates from Lake Manyara, Tanzania (Stifler, 1941) and *C. octosporus* Blackwell & Gilbertson recorded on *Tenuirostermes tenuirostris* (Desueau) from Mexico (Blackwell & Gilbertson, 1981). The former species was also described on *M. michaelseni* (Sjostedt) and *M.* cf. *subhyalinus* (Rambur) alates from Kenya (Blackwell & Gilbertson, 1984). The above species represent the few known examples of exclusive fungal pathogen occurrence on termites.

Toft *et al.* (unpubl. reports) found that termite damage to crops, trees and buildings was more severe in low to mid-altitude semi-arid and arid areas of Kenya. The above reports led to the planning of an exploratory survey in selected termite-prone areas, to identify naturally occurring entomopathogenic fungi on termite species, as potential biological control agents.

Systematic laboratory and field-based investigations on a naturally occurring, termite-specific fungal pathogen were also initiated, to contribute to the scanty knowledge of such fungal pathogen species.

MATERIALS AND METHODS

Natural occurrence of fungal pathogens on termites in Kenya

The survey for fungal pathogens on termites was between 1991 and 1993, during the "long-rains season" (March to May) and "short-rains season" (November to December). Selected sites covered a specific range of termite-prone ecological zones; Isiolo district, an arid area in North Eastern Province of Kenya; Kajiado district, a semi-arid area in South-eastern Rift Valley Province of Kenya and Siaya district, a wet and humid area in Nyanza Province, Western Kenya.

The basic survey procedure was to visually inspect termite habitats thoroughly, for termite cadavers having apparent mycosis symptoms such as internal mummification or fungal growth. The search for termite cadavers at specific termite habitats was general; the underside of stones and logs, tree stumps, the vicinity of termite mounds, subterranean termite nests, infested farms and forestry nurseries were some of the places inspected during the survey. Infected and non-infected termite cadavers were collected by hand or by means of fine forceps. All termite cadavers were brought back to the laboratory for further investigation. Even termite worker, soldier and alate cadavers from laboratory populations were routinely examined to detect fungal infection.

After the exploratory survey, a monthly alate sampling/collection and alate flight monitoring schedule (November 1993-April 1995) was initiated to investigate the occurrence of a termite-specific fungal pathogen on *M. subhyalinus* alates. A site, situated eight km. South-east of Kajiado town (grid reference: 1°52.5"S 35°53"E: altitude 1500 metres) was selected for the field studies.

The basic sampling procedure was to look for mummified and non-mummified termite dealates, mainly by turning over stones, within a 300-metre radius of a marked mature *M. subhyalinus* mound. An upper limit of 200 stones was set for dealate sampling and collection. Back in the laboratory, mummified dealate cadavers were

dissected to confirm mycosis and to isolate fungus but non-mummified ones were incubated to observe mycosis. Cadavers of dealates and alates originally collected live during the monthly sampling and/or actual flights were incubated or dissected to observe mycosis.

Diagnosis and morphological structure of fungal pathogen isolates

Tentative generic fungal diagnoses on termite hosts and *in vitro* were attempted before mummified dealate specimens and *in vitro* fungal cultures were sent to the International Institute of Biological Control (IIBC), United Kingdom (UK) and the Centraalbureau von Schimmelcultures (CBS), The Netherlands, for confirmation of the preliminary diagnoses. Light and electron microscopic photography was used to document the diagnostic teleomorph and anamorph structures of the fungus.

***In vitro* isolation and culture of fungal pathogens from termite hosts**

Fungal isolates were derived from field-collected, infected dealates according to standard methodology (Poinar & Thomas, 1984), on three isolation media, Potato dextrose agar (PDA), Sabouraud dextrose agar (SDA) and Molisch agar (MoA). Different mycological media were tested for fungal isolate culture and maintenance. They included solid media (PDA, malt-agar (MA), SDA, SDA egg-yolk agar (SDAY+EY), Sabouraud maltose agar (SMA), semi-synthetic medium (SSM)) and liquid media (SDA, SMA, MoA).

RESULTS AND DISCUSSION

Natural occurrence of fungal pathogens on termites in Kenya

Exploratory survey results show that 20 mummified *M. subhyalinus* dealate specimens were collected near Isiolo town (Table 1) during the 1991 "short-rains season". They were subsequently confirmed to be infected with *C. bisporus* (teleomorph). During the 1993 "short-rains season", 17 mummified *M. subhyalinus* dealates, infected with *C. bisporus* (anamorph), near Kajiado town (Table 1).

At the Kajiado field site, 15 freshly infected mummified *M. subhyalinus* dealate cadavers, without full blown *C. bisporus* infection symptoms, were sampled within days of alate flights during the 1994 "short-rains season" (November and December) (Table 1). Five mummified dealate cadavers sampled at the site in March 1995, had full blown mycosis symptoms (Table 1), having been presumably infected during 1994 "short-rains season". Similar symptoms were observed on dealate cadavers collected during the 1993 "short-rains season", after an unspecified pathogen incubation period. Under field conditions, it is probable that the incubation period of *C. bisporus* in *M. subhyalinus* hosts takes at least four weeks.

No alates flew at the Kajiado site during the period December 1993-October 1994. Consequently, no *M. subhyalinus* dealate cadavers were collected. Two alate flights (one in mid-November and the second in early December) were recorded at the site, during the 1994 "short-rains season". There were no subsequent flights at the site up to the end of the sampling programme in April 1995. *M. subhyalinus* and *M. michaelseni* alate flights in the Kajiado area have been respectively reported by Blackwell & Gilbertson (1984) and Darlington (1986).

Observations from the exploratory survey and the field sampling showed a correlation between "short-rains season" and the occurrence of *C. bisporus* on termite alates. It was also noted that dealate cadavers were collected only beneath stones.

Diagnosis and morphological structure of fungal pathogens from de-alate specimens

C. bisporus isolates derived from Isiolo specimens had the typical teleomorph structures (perithecia, asci and ascospores) similar to those described from *C. bisporus* on *M. natalensis* by Stifler (1941) and on *M.* cf. *subhyalinus* and *M. michaelseni* by Blackwell and Gilbertson (1984). *C. bisporus* isolates derived from Isiolo specimens (present studies) had the diagnostic paired ascospores. *C. bisporus* anamorph on Kajiado specimens is *Hymenostilbe* sp., probably a new species (Samson, pers. comm.) while that on Isiolo specimens is *Hirsutella* sp. (Evans, pers. comm.). However, *Hirsutella* and *Hymenostilbe* are anamorphs of *Cordyceps*, which is taxonomically distinct from *Cordycepioideus* (Evans, pers. comm.). Blackwell & Gilbertson (1984) linked *Cordycepioideus* spp. to *Cordyceps militaris* (Fr.) Link but they have no formal descriptions of the anamorph form for *C. bisporus* and *C. octosporus*.

Light microscopic photographs and electron micrographs were taken of *C. bisporus* teleomorph structures (perithecia and ascospores) and anamorph structures (conidiophores and conidia).

***In vitro* isolation and culture of *C. bisporus* from termite hosts**

C. bisporus isolates derived from termite alate hosts are shown in Table 2. *In vitro* culture of *C. bisporus* teleomorph isolates was possible on all solid (SDA, SMA, PDA, SDA+EY, MA and SSM) and liquid (SDA, SMA and MoA) media tested. Synnemata growth was predominant on solid media while mycelium growth was more evident in liquid media. Ascospores were observed on all solid media except MA. The best media for culturing *C. bisporus* teleomorph were SDA, SDA+EY and MoA. In the above media, synnemata and ascospores were consistently observed in at least one *C. bisporus* teleomorph isolate (KVL 606). Simple, sometimes branched synnemata were regularly observed in *C. bisporus* anamorph isolates (KVL 607, 623, 624, 627, 628) on SDA, SDA+EY and MoA. However, *C. bisporus* ascospores were neither associated with infected dealate specimens collected from Kajiado nor with *in vitro* cultures of *C. bisporus* anamorph isolates.

Blackwell & Gilbertson (1984) failed in attempts to germinate *C. bisporus* ascospores (teleomorph) from termite hosts, *in vitro* but managed to get an undescribed anamorph isolate from conidia in culture, which they linked to *Cordyceps militaris* .

Conclusion:

The present studies have contributed significantly to the scanty knowledge of naturally occurring termite pathogens by identifying the fungal pathogen (*C. bisporus*), from an exploratory survey. *C. bisporus* was isolated from infected termite hosts and maintained in culture on a range of mycological media. Important diagnostic structures of *C. bisporus* were documented by means of electron microscopy for the first time ever and these studies are on-going. The field studies on the occurrence of *C. bisporus* on termite alates were the first of their kind and could serve as a basis for future studies on *Cordycepioideus* spp.

ACKNOWLEDGEMENTS

The studies were funded by Danish International Developmental Assistance (DANIDA) through a collaborative research project between Kenya Agricultural Research Institute (KARI) and Danish Technological Institute (DTI). Mr. Charles Dewhurst is acknowleged for the collection of infected termite alate specimens from Isiolo, Kenya. Drs. Chris Prior and Harry C. Evans, International Institute of Biological Control (IIBC), United Kingdom (UK), are acknowledged for fungal isolation from termite alate hosts and identification and Dr. Robert A. Samson, Centraalbureau von Schimmelcultures (CBS), The Netherlands, for fungal identification. Technical assistance by Dorte Berthelsen, Bodil Jørgensen and Lars Heegaard Jensen at RVAU, Denmark, is gratefully acknowledged. Peter Karanja, Elijah Nyamwange, Veronica Mirie and Lucy Karanja provided technical assistance at NARC, Muguga, Kenya. Mr. Joseph K. Kamau collaborated in termite alate flight monitoring and live alate collection at Kajiado.

REFERENCES

Blackwell, M. & Gilbertson, R. L. (1981) *Cordycepioideus octosporus*, a termite suspected pathogen from Jalisco, Mexico
Mycologia 73:358-362.

Blackwell, M. & Gilbertson, R. L. (1984) New information on *Cordycepioideus bisporus* and *Cordycepioideus octosporus*
Mycologia 76 (4):763-765.

Darlington, J.P.E.C. (1986) Seasonality in mature nests of the termite *Macrotermes michaelseni* in Kenya
Insectes Sociaux 33(2):168-169.

Poinar, G.O. & Thomas, M. G. (1984) Laboratory guide to insect pathogens and parasites
Plenum Press New York pp. 89-91.

Stifler, C. B. (1941) A new genus of Hypocreales
Mycologia 33:82-86.

Table 1: Natural occurrence of fungal pathogens on termite alate hosts in Kenya

Date & locality of collection	Pathogen species Host species Form	Total collection points	Total alates	Total live alates	Total dead alates	Total alates with fungus
December 1991 Isiolo	*C. bisporus* *M.subhyalinus* Teleomorph	1	20	0	20	20
November 1993 Kajiado	*C. bisporus* *M.subhyalinus* Anamorph	5	17	0	17	17
November 1994 Kajiado	*C. bisporus* *M. suhyalinus* Anamorph	12	32	7	25	7
December 1994 Kajiado	*C. bisporus* *M.subhyalinus* Anamorph	6	12	1	11	8
March 1995 Kajiado	*C. bisporus* *M.subhyalinus* Anamorph	1	5	0	5	5

Table 2: *C. bisporus* isolates obtained from termite alate hosts in Kenya

Locality of specimen collection	Date of isolation	Host species Pathogen species Isolate	Isolation medium*
Isiolo	October 1992	*M.subhyalinus* *C. bisporus* KVL 605	MoA
Isiolo	December 1993	*M.subhyalinus* *C. bisporus* KVL 606	MoA
Isiolo	January 1995	*M.subhyalinus* *C. bisporus* KVL 625	PDA
Kajiado	April 1994	*M.subhyalinus* *C. bisporus* KVL 607	MoA
Kajiado	January 1995	*M.subhyalinus* *C. bisporus* KVL 623	MoA
Kajiado	January 1995	*M.subhyalinus* *C. bisporus* KVL 624	SDA
Kajiado	January 1995	*M.subhyalinus* *C. bisporus* *KVL 627*	SDA
Kajiado	January 1995	*M.subhyalinus* *C. bisporus* *KVL 628*	SDA

* All isolations were made under ambient laboratory conditions.

EFFICACY OF ENTOMOPATHOGENIC FUNGUS *PAECILOMYCES FUMOSOROSEUS* AGAINST WHITEFLY (*TRIALEURODES VAPORARIORUM*) IN GREENHOUSE TOMATO CULTURES

D.Sosnowska, J.Piatkowski
Department of Biological Methods of Pest Control and Quarantine, Institute of Plant Protection, Miczurina 20, 60-318 Poznań, Poland

Summary

The new biological insecticide based on highly effective, naturally occurring strain of fungus *Paecilomyces fumosoroseus* (PFR) was evaluated. The presently reported study has been conducted in three commercial greenhouses in Siechnice and Krzeszowice in 1994, and Wloszakowice in 1995. The highest mortality reaching (92%) was shown in the treatment with PFR applied at the concentration 0,4% (Siechnice greenhouse). In Krzeszowice the fungus was applied once only at 0,2% concentration. This treatment caused 74% mortality. In Wloszakowice PFR was applied as a corrective factor with *Encarsia formosa*, and together they reduced whitefly larvae population from 180 alive to 13 alive per tomato leaf at the end of experiment.

Introduction

Greenhouse whitefly (GWF) (*Trialeurodes vaporariorum*) still remains the main insect pest of greenhouse crops, including tomato. Recent development of integrated methods of pests control and proecological approach to plant cultivation (e.g.use of natural pollination) require a new, alternative agents to control this pest. Research conducted worldwide revealed that the entomopathogenic fungus *Paecilomyces fumosoroseus* (PFR) can be one of such alternative.This fungus occurs in many parts of the world where it plays an important role in the natural control of many insects (Smith, 1993).

An isolate of PFR has been isolated in Florida (USA) by Dr Lance Osborne, and strain PFR-97- Apopka is currently being evaluated in the laboratory and under greenhouse conditions in biological control against whiteflies (Osborne, Landa, 1992). The fungal biopreparation based on PFR has been developed by W.R.Grace Company (USA).

This paper reports experiments in three commercial greenhouses in Poland, which evaluated the efficacy of PFR against GWF on tomato cultures.

Material and Methods.

Commercial greenhouse in Siechnice (West of Poland).

The fungus was applied twice in weekly intervals during the spring time. The size of plots were 250 m^2 per treatment. The following treatments of PFR were evaluated: 0,1%; 0,2% and 0,4%. Applaud (buprofezina) in 0,15% concentration was used as an chemical insecticide to compare. Three randomly selected leaves per plant (from top, middle, bottom) were examined for each sample in the laboratory and the number of alive and dead larvae was counted and recorded. First assessment was made week after the first spray, second assessment was made week after second spray.

Commercial greenhouse in Krzeszowice (South of Poland).

The fungus was applied once only at 0,2% concentration during the autumn time. PFR was compare with mixture of chemical insecticides: Lannate (metomyl) (0,15%) + Propotox (propoksur+metoksychlor) (0,3%).

Commercial greenhouse in Wloszakowice (West of Poland).

800 m^2 of tomato plants were sprayed three times with PFR (0,1%) in weekly intervals. Before PFR spray parasite chalcidoid wasp - *Encarsia formosa* (5 Encarsia/plant) were used.

Results and Discussion

In Siechnice greenhouse the highest mortality reaching 92% was shown in the treatment with PFR applied at the concentration 0,4%. Applaud was not effective against greenhouse whitefly (Fig.1).

Fig.1. Mortality of GWF larvae on tomato plants (Siechnice, 1994)

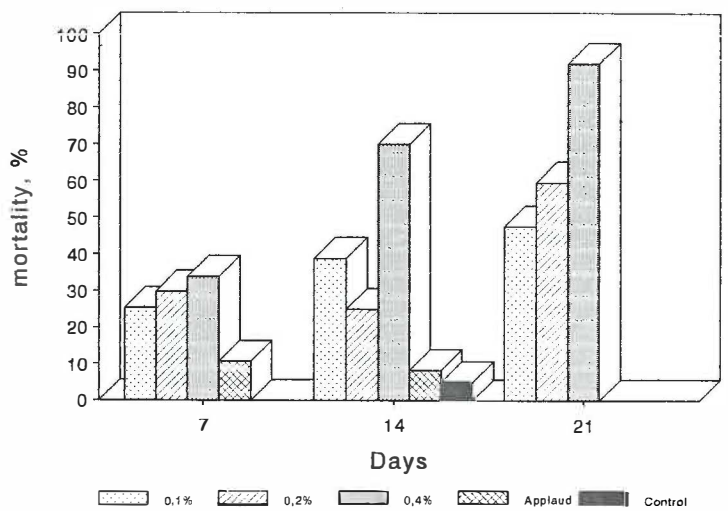


Fig.2.Mortality of GWF larvae on tomato plants (Krzeszowice, 1994)

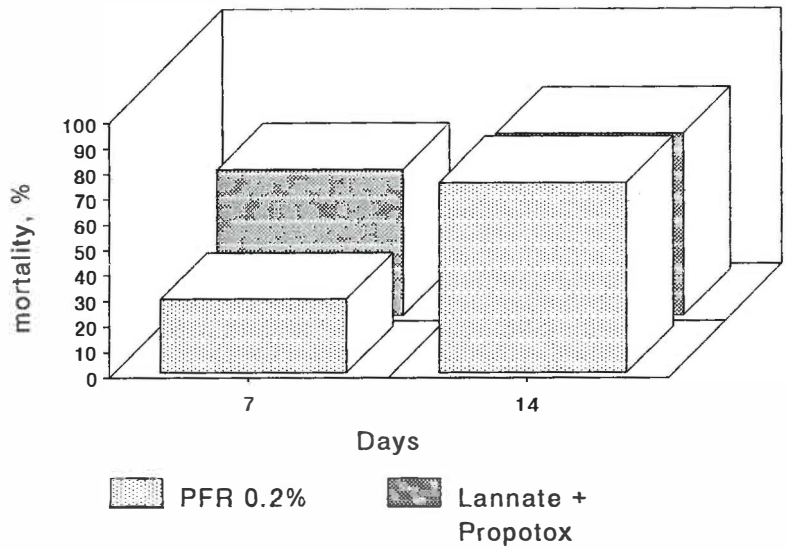
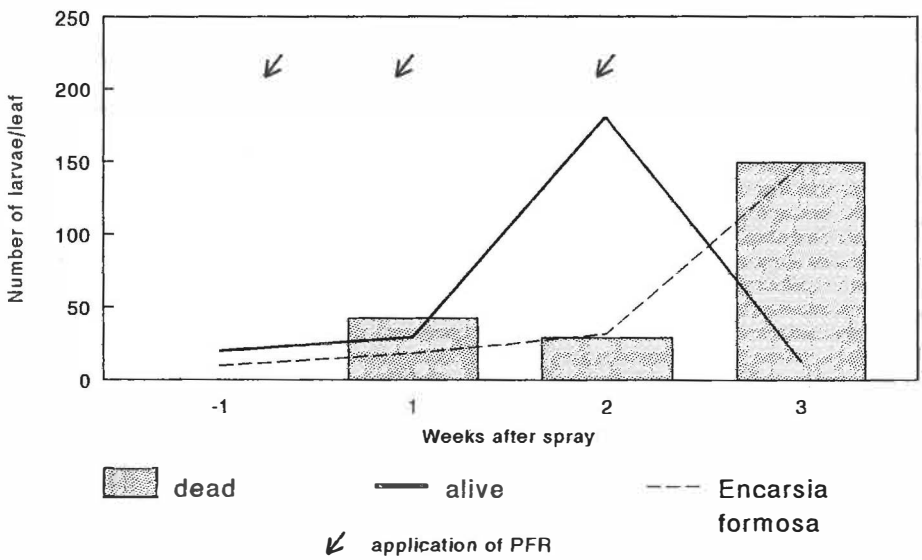


Fig.3. Develop of whitefly population after use of PFR as a corrective factor



In Krzeszowice PFR treatment caused 74% mortality on second week after spray, what we did not observe in Siechnice in the same treatment (Fig.2). Significant influence on PFR efficacy have season environmental conditions. In Krzeszowice the experiment was conducted during the autumn time, where humidity was higher than in Siechnice during the spring time.

Significant reduction of whitefly population was observed in Wloszakowice, where fungus was applied as a corrective factor with *Encarsia formosa*. Both biological agents are compatible and together reduced whitefly larvae population from 180 alive to 13 alive per tomato leaf at the end of experiment (Fig.3). The number of *Encarsia formosa* and number of dead GWF larvae increased. *Encarsia formosa* and PFR may result in complementary mortality of whitefly as we can see on Fig.3.

Conclusions

- PFR may be one of alternative biological control agents for greenhouse pests control during the spring and autumn time.
- The obtained results showed that PFR could be applicated as a corrective factor together with *Encarsia formosa*.

References.

OSBORNE, L.S., LANDA, Z. 1992. Biological control of whiteflies with entomopathogenic fungi. Florida Entomologist 75(4): 457-470.

SMITH, P. 1993. Control of *Bemisia tabaci* and the potential of *Paecilomyces fumosoroseus* as a biopesticide. Biocontrol News and Information 14 (4): 71-78.

EFFECT OF *BEAUVERIA BASSIANA* AGAINST OVERWINTERING PEA LEAF WEEVIL, *SITONA LINEATUS*.

T. STEENBERG[1,3] & H.P. RAVN[2]

[1] Royal Veterinary and Agricultural University, Section of Zoology, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark

[2] Institute of Plant and Soil Science, Lottenborgvej 2, DK-2800 Lyngby, Denmark

[3] Present affiliation: Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Lyngby, Denmark

Abstract

The entomopathogenic fungus *Beauveria bassiana* was tested in a semifield experiment against adult pea leaf weevils (*Sitona lineatus)* overwintering in buckets with lucerne, white clover or barley straw. Inoculum of *B.bassiana* strain 195, originally isolated from the homologous host, was grown on crushed wheat kernels and applied at a concentration of 13 g per bucket corresponding to approx. 6.8 x 10^5 spores per g soil. The experiment was started on 21 September and at the end of overwintering (late April), mortalities were 99.5 % in lucerne, 94% in white clover and 100% in buckets with barley straw.. In buckets with barley straw, mortality was due to starvation as weevils apparently were not fully fed for overwintering. Data from buckets collected at the end of October showed that weevils had been infected but mortality was postponed until the onset of spring. Natural winter mortality in control buckets reached 64.7% (lucerne), 51.0% (clover) and 87.4% (straw).

Key-words Insects, pea leaf weevil, Sitona lineatus, winter mortality, microbial control, entomopathogenic fungi, *Beauveria bassiana*

Introduction

The pea leaf weevil *Sitona lineatus* L. (Col., Curculionidae) is an important pest of leguminous crops in many parts of the World . In Denmark, the pest status of *S. lineatus* has increased within the last 10-15 years due to a substantial increase in the pea growing area. Adult weevils overwinter in perennial legumes such as lucerne and clovers, or in long grass, cereal stubble etc. After migration to annual legume crops in spring, weevils mate and deposit eggs. Larvae feed on the nitrogen fixating root nodules. Teneral adults emerge in late July and August, and migrate to overwintering sites after wilting or harvesting of the pea crop. Previous attempts using entomopathogenic fungi for microbial control of this insect or closely related species have mainly been directed against young instars in annual crops (Müller-Kögler & Stein, 1970; Bailey & Milner, 1985; Verkleij et al., 1992). However, adults are very susceptible to *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) under natural conditions and epizootics have been described (Hans, 1959; Fisher, 1977). After selection of an efficient strain of *B. bassiana* isolated from the homologous host, we conducted a semi-field experiment in order to evaluate the

control potential of the fungus when applied against overwintering *Sitona lineatus* in autumn. This summary paper presents basic results from the experiment.

Materials and methods

For ease of application, inoculum of *Beauveria bassiana* strain 195 was produced on autoclaved crushed wheat and applied to buckets with soil and three different types of plant cover (lucerne, white clover and barley straw). On 21 September 1993 inoculum was applied to the soil surface at a concentration of 13 g wheat kernels per bucket, corresponding to approx. 6.8×10^5 conidia per g soil. Most likely the fungus proliferated further on the kernels after application thus increasing the fungus titer substantially. 50 field collected weevils were applied to each of 30 replicate buckets per treatment. For each plant type 30 untreated control buckets were set up. At six times during winter, five buckets per treatment were removed by randomn sampling , sampling commencing at 28 October and ending at 26 April. After hand sorting of the buckets they were flotated and dead and live weevils were removed. Dead specimens were incubated in moist chambers and live weevils were surface disinfected in NaOCl (1%, 1 min.) followed by rinsing twice in sterile water and subsequently incubated separately in 30 ml plastic cups with a clover leaf for 2 weeks. Weevil mortality and sporulation rate in the field and after laboratory incubation was determined.

Results and discussion

Mean final mortality at the last sampling date in late April was 99.5% in lucerne, 94% in clover and 100% in straw. Of the very few surviving weevils, all proved to be infected by fungus and eventually would have succumbed to disease in the field. However, already in late October almost 100% of live weevils sampled from lucerne and clover proved to be infected although not yet dead. At this time, significant more weevils had died in buckets with barley straw. In October, mean fresh weight of surviving weevils from these buckets was considerably lower than the corresponding weight of weevils from buckets with lucerne or clover, thus indicating that even at the end of September weevils were not yet fully fed and ready for overwintering. Thus the high mortality found in barley straw most likely was due to starvation.

Natural mortality in control buckets in late April was 64.7% in lucerne, 51.0% in clover and 87.4% in barley straw and was partly due to infection by *B. bassiana.* This support previous danish field studies on the natural impact of *Beauveria bassiana* on *Sitona* weevils overwintering in lucerne (Steenberg, 1993), where the fungus was found to infect 70% of weevils overwintering in multiple-year lucerne. The apparent fungus prevalence (cadavers sporulating in the field) remained low throughout the winter, even though an increase in % sporulation was observed in spring.

The strategy of controlling insect pests with entomopathogens applied prior to overwintering in order to reduce spring emergence has only rarely been explored. However, Gaugler et al. (1989) employed the strategy using *Beauveria bassiana* to control overwintering Colorado potato beetle *Leptinotarsa decemlineata* Say but did not succeed in reducing spring emergence even though a high proportion of beetles proved to be infected at the time of emergence. In contrast, strain 195 of *B. bassiana* increased winter mortality of *Sitona lineatus* in lucerne and clover to 94% or more when applied at the end of

September. Dose-response experiments using inoculum on wheat kernels should be initiated in order to evaluate the actual inoculum levels required in this type of application strategy. In conclusion, the selected strain has excellent control potential against overwintering weevils at the inoculum concentration investigated. We do not, however, consider fungus treatment of large areas of perennial leguminous crops realistic, and future use of *B. bassiana* strain 195 against pea leaf weevil therefore should integrate the pathogen with efficient methods for concentration of the adult life stage, e.g. trap crops such as lucerne, vetch or winter peas, or traps baited with aggregation pheromones (Blight et al., 1991).

References

BAILEY, O. & MILNER, R., 1985. *Sitona discoideus*: A suitable case for control with pathogens? Proceed.4th Australasian Conf.Grassl.Invert.Ecol. Canterbury, New Zealand, My 1985, 210-216.

BLIGHT, M.M., DAWSON, G.W., PICKETT, J.A. & WADHAMS, L.J., 1991. The identification and biological activity of the aggregation pheromone of *Sitona lineatus*. Aspects of Applied Biology 27, 137-142.

FISHER, J.R., 1977. The population dynamics of the pea leaf weevil *Sitona lineatus* (L.) in northern Idaho and eastern Washington. Ph.D.-thesis, University of Idaho, 180 pp.

GAUGLER, R., COSTA, S.D. & LASHOMB, J., 1989. Stability and Efficacy of *Beauveria bassiana* Soil Inoculations. Environ.Entomol. 18(3), 412-417.

HANS, H., 1959. Beiträge zur Biologie von *Sitona lineatus* L. Z.angew.Entomol. 44, 343-386.

MÜLLER-KÖGLER, E. & STEIN, W., 1970. Gewächshausversuche mit *Beauveria bassiana* (Bals.) Vuill. zur Infektion von *Sitona lineatus* (L.)(Coleopt., Curcul.) im Boden. Z.angew.Entomol. 65(1), 59-76.

STEENBERG, T., 1993. Natural impact of entomopathogenic fungi on *Sitona* weevils. Tidsskrift for Planteavl. Specialserie SS-2237, 145-152 (in Danish, English abstract).

VERKLEIJ, F.N., VAN AMELSVOORT, P.A.M. & SMITS, P.H. , 1992. Control of the pea weevil (*Sitona lineatus* L)(Col., Curculionidae) by the entomopathogenic fungus *Metarhizium anisopliae* in field beans. J.Appl.Ent. 113, 183-193.

LABORATORY EVALUATION OF *BEAUVERIA BASSIANA* (BALS.) VUILL. AGAINST THE BARK BEETLE, *IPS TYPOGRAPHUS* (L.)(COLEOPTERA, SCOLYTIDAE).

Rudolf WEGENSTEINER

Institute of Forest Entomology, Forest Pathology and Forest Protection, University of Agriculture-Vienna, Hasenauerstraße 38, A-1190 Vienna, Austria.

1. Introduction

The insect pathogenic fungus *Beauveria bassiana* can be found in different environments as a pathogen of many insect species. It also occurs in the bark beetle *Ips typographus*, an important pest on spruce in central and northern Europe. Occasioully *B. bassiana* is relatively abundant in *I. typographus*-field populations (Balazy, 1966; Mills, 1983; Wegensteiner et al., in press). First tests with the Czech *B. bassiana*-spore preparation "Boverol" against *I. typographus* provided high fungus infection rates (Matha & Weiser, 1985; Wegensteiner, 1992). Abiotic factors (temperature, relative humidity) are known to play a key role in the efficacy of insect pathogenic fungi (ref. in Müller-Kögler, 1965; in Feng et al., 1994). The permanent problems with *I. typographus* and the lack of efficient control methods were the reason to examinate the efficacy of a *B. bassiana* spore preparation at distinct spore doses, inoculation modes and temperatures in this bark beetle species more exactly.

2. Material and Methods

Adult bark beetles fresh collected from trap trees and pheromone-baited traps were inoculated either by dipping in different concentrations of *B. bassiana*-spore powder (10^{10}, 10^9, 10^8 or 10^7 conidia/g) or -spore suspension (7,5x10^7conidia/ml or 3x10^7conidia/ml) for 10 sec.. In an additional experiment healthy bark beetles were placed on spruce-bark pieces (4 x 5cm) treated with spore powder (3x10^7conidia/cm^2) or spore suspension (3x10^6conidia/cm^2). The conidiospores had been produced in the Czech Republic (*B. bassiana*-strain 90311/48) by the method of Samsinakova et al. (1981) and were finally formulated and adjusted by former Wacker-Dow company (Munich, Germany) which supported this study. The beetles in the control groups were either treated with the formulation additives (Talkum N), dipped in an 0.1% aqueous Tween 80 solution (10 sec.), or were totally untreated. The beetles of each variant were incubated with some spruce bark pieces at 20°, 25° or 30°C, without light, and at 93.5% to 91.0% relative humidity using sat. potassium nitrate. Mortality was recorded daily till the death of the last beetle. The mortality data of *I. typographus* of the whole experiments were used for a homogeneity analysis according to Kolmogoroff and Smirnoff. The LT_{50} of those beetles were calculated which evidently died by fungus infection. The pathogenic efficacy was calculated as a percentage of the total number of beetles and corrected according to the formula of Schneider-Orelli (Schneider Orelli, 1981) a version of Abott's-formula.

3. Results

3.1 Beetle mortality after inoculation with spore powder

Beetle inoculation with the highest *B. bassiana*-spore-powder dose (10^{10} con./g) yielded shortest LT_{50} values and highest infection rates in *I. typographus* at all three temperatures. The LT_{50} values in the 10^9- and 10^8-variants were two to six times longer than in the 10^{10}-variant, and infection rates were lower about twenty to fourty percent (one exception: 10^9 con./g at 30°C).

In the lowest concentration at 25°C infection rate did not reach even 50% (Table 1).

Table 1: LT_{50} values (in days) and mortality by mycosis (in %) in *I. typographus* (n) after beetle inoculation with four *B. bassiana*-spore powder concentrations and a control (% spontaneous infection) at three temperatures (20°, 25° and 30°C).

		10^{10}	10^9	10^8	10^7	control
20°	LT_{50}	6.6	15.0	11.1	25.3	20.5
	%	100.0	68.8	83.8	65.3	21.4
	n	125	110	110	110	98
25°	LT_{50}	4.4	17.0	12.5	-	15.5
	%	98.9	59.0	62.3	30.0	20.2
	n	106	104	103	98	94
30°	LT_{50}	3.7	12.8	24.6	23.3	20.1
	%	81.2	94.2	66.4	64.8	3.7
	n	105	108	111	112	81

Progress of mortality was fastest at the highest concentration at all temperatures ($p < 0.001$). The beetles of the control groups (treated with Talkum N) lived significantely longer than the inoculated variants with exception of the 10^7-variants at all temperatures and the 10^8-variant at 30°C.

3.2 Beetle mortality after inoculation with spore suspension

The LT_{50} values of the beetles inoculated with the lower spore suspension (3.0×10^7 conidia/ml) were lower in most cases. Only at 20°C the higher suspension dose reached a lower LT_{50} value and lead to the highest beetle-infection rate within the distinct suspension-variants. Infection rates were lower at 25° and 30°C in comparison with 20°C. A high spontaneous infection rate could be observed in the beetles of the 25°C control group (Table 2).

Table 2: LT_{50} values (in days) and mortality by mycosis (in %) in *I. typographus* (n) after beetle inoculation with two *B. bassiana*-spore suspensions and a control (% spontaneous infection) at three temperatures (20°, 25° and 30°C).

		7.5×10^7	3.0×10^7	control
20°	LT_{50}	7.8	10.1	19.4
	%	94.5	86.9	19.8
	n	114	143	111
25°	LT_{50}	13.0	9.4	19.2
	%	66.0	78.9	29.7
	n	110	145	111
30°	LT_{50}	9.9	9.8	19.6
	%	86.0	76.5	10.3
	n	111	142	116

No significant differences could be found in mortality progress between the beetles inoculated with the higher or lower suspension dose at all three temperatures.

3.3 Mortality of beetles in contact with spore powder or spore suspension treated bark

The LT_{50} values of untreated beetles subjected to permanent contact with spore powder- or spore suspension-treated bark pieces were lower in the variants with spore powder treatment at 20°

and 25°C, whereas at 30°C the spore suspension treated variant had the lower LT_{50} value. The infection rates were high in both variants, with exception of the 25°C spore powder treated bark-variant. The beetles in the 20°C-control had an inexplicably high rate of spontaneous infection (Table 3).

Table 3: LT_{50} values (in days) and mortality by mycosis (in %) in *I. typographus* (n) having permanent contact with spore powder-(b+p; $3x10^7$) or spore suspension-(b+s; $3x10^6$) treated bark (conidia/cm²) and an untreated control (% spontaneous infection) at three temperatures (20°, 25° and 30°C).

		b+p	b+s	control
20°	LT_{50}	5.7	6.2	11.8
	%	90.9	97.0	36.5
	n	104	108	85
25°	LT_{50}	2.6	5.0	22.5
	%	68.0	93.5	14.0
	n	109	108	100
30°	LT_{50}	9.0	8.2	7.9
	%	97.0	92.6	11.0
	n	110	106	91

Within a specific treatment, mortality course of beetles was fastest at 25°C in comparison with 20° and 30°C ($p< 0.001$) and faster at 20°C than at 30°C ($p< 0.001$). The higher dose on the spore powder treated bark was significantely better effective only at 25°C than the lower spore suspension dose ($p< 0.001$). The mortality progresses in the treated variants were faster at 20° and 25° than in the beetles of the control groups ($p< 0.001$) but at 30°C no statistic differences could be found ($p> 0.05$).

4. Discussion

The *B. bassiana* spore preparation was shown to have a good virulence on *I. typographus*. The initial concentration of the spore powder (10^{10} conidia/g) is obviously an exorbitant high dose that individual distinctions in vitality play a negligible role as evidenced in small temperature-dependent divergences of values. On the other hand, the relatively high LT_{50} values and the lowest infection rates in the lowest spore powder concentration (10^7 conidia/g) show the importance of conidia concentration. But these results indicate also that *I. typographus* individuals are able to counter *B. bassiana* depending most probably on the individual potency and actual fitness of the bark beetles respectively on the dominant temperature. However, infection rate was confirmed to be a function of inoculation dose but independant of temperature, similar to the results Wulf (1983) found in *P. chalcographus*. Best effects could be found at 20° and 25°C, well known as preference temperature of *B. bassiana*; 30°C had no negative consequences for infection success but *I. typographus* may have been adversely affected. Apart from that the range from 20° to 30°C is generally recognized as being tolerable for both *I. typographus* (Postner, 1974) and *B. bassiana* (ref. in Feng et al., 1994).
It is conspicuous that the highest infection rates could be found in the treated bark-variants and in the suspension-variants, neglecting only the highest spore powder-variant (10^{10} conidia/g) at all three temperatures and the 10^9 powder-variant at 30°C. These results confirm earlier observations about the great importance of relative humidity and of contact humidity for conidia germination (ref. in Feng et al., 1994). As shown in the treated bark-variants a concentration of $3x10^6$ conidia/cm² bark seems to be a sufficient infection dose to kill more than 90% of beetles. Furthermore, statistical

comparison of beetle mortality generally demonstrated the superiority of both treated bark-variants especially at 20° and 25°C. Wegensteiner (1992) was also able to observe highest *B. bassiana*-infection rates in *I. typographus* as well as very short mean life spans after only a brief period of beetle contact with treated bark pieces (1 min. to 24 h). The great discrepancy between laboratory and field tests known also from experiments with distinct entomopathogenic fungus species (ref. in Bathon, 1991; Tanada & Kaya, 1993) forces to continue these investigations under field conditions. It is expected that mortality will be delayed and infection rates will be lower under field conditions than in the laboratory experiments. This fact is of eminent importance from the practical point of view for selecting application strategies in field.

The relatively high *B. bassiana*-infection rates in some of the control groups, and the high prevalence of *B. bassiana* as reported from *I. typographus*-field populations (Wegensteiner et al., in press) emphasize this entomopathogenic fungus species. Selecting an isolate with highest virulence against *I. typographus* is presumed to have good chances to control this bark beetle by the way of augmentation via bark beetle carriers starting from a treated trap tree or from a modified trap (Wulf, pers. comm.). However, special attention must be paid on application strategies because of unspecific effect of *B. bassiana* against a great number of distinct, beneficial and indifferent insect species.

References

BALAZY, S., 1966: Living organisms as regulators of population density of bark beetles in spruce forest with special reference to entomogenous fungi. I. Poznan.Tow.Przyj.Nauk Wydz. Nauk Roln.Lesn., Pr.Kom.Nauk Roln.Kom.Nauk Lesn. **21**, 3-50.

BATHON, H., 1991: Möglichkeiten der biologischen Bekämpfung von Borkenkäfern. In: Borkenkäfer-Gefahren nach Sturmschäden. WULF, A., R.KEHR (Eds.), Mitt.Biolog. Bundesanst. f. Land- u. Forstwirtschaft Berlin-Dahlem, Heft **267**, 111-117.

FENG, M.G., T.J. PODRAWSKI, G.G. KHACHATOURIANS, 1994: Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for Insect control: current status. Biocontrol Science and Technology **4**, 3-34.

MATHA, V., J. WEISER, 1985: Effect of the fungus *Beauveria bassiana* on adult bark beetles *Ips typographus*. Conf.Biol.Biotechn.Contr.Forest Pests 1985, Tabor (CSFR).

MILLS, N.J., 1983: The natural enemies of scolytids infesting conifer bark in Europe in relation to the biological control of *Dendroctonus spp.* in Canada. CIBC Biocontrol News Info. **4**, 305-328.

MÜLLER-KÖGLER, E., 1965: Pilzkrankheiten bei Insekten. P. Parey, Berlin and Hamburg.

POSTNER, M., 1974: Scolytidae (=Ipidae) Borkenkäfer, in Die Forstschädlinge Europas Bd. 2: Käfer (SCHWENKE, W., Ed.) P. Parey, Hamburg and Berlin, pp. 334-487.

SAMSINAKOVA, A., S. KALALOVA, V. VLCEK, J. KYBAL, 1981: Mass production of *Beauveria bassiana* for regulation of *Leptinotarsa decemlineata* populations. J.Invertebr. Pathol. **38**, 169-174.

SCHNEIDER-ORELLI, 1981: Manual für Feldversuche im Pflanzenschutz. Documenta CIBA-GEIGY (W. PÜNTENER, Ed.) pp. 33-34. Agro-Division, CIBA-GEIGY AG, Basel, Switzerland, 2.Aufl..

TANADA, Y & H.K. KAYA, 1993: Insect Pathology. Academic Press.

WEGENSTEINER, R., 1992: Untersuchungen zur Wirkung von *Beauveria*-Arten auf *Ips typographus* (Col., Scolytidae). Mitt.Dtsch.Ges.allg.angew.Ent. **8**, 104-106.

WEGENSTEINER, R., J. WEISER, E. FÜHRER, in press: Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Coleoptera, Scolytidae). J.Appl.Ent..

WULF, A., 1983: Untersuchungen über den insektenpathogenen Pilz *Beauveria bassiana* (Bals.) Vuill. als Parasit des Borkenkäfers *Pityogenes chalcographus* L. (Col., Scolytidae). Z.angew Ent. **95**, 34-46.

EFFECTS OF DESTRUXINS ON *PIERIS BRASSICAE* AND *AGROTIS SEGETUM*.

L. THOMSEN, J. EILENBERG, P. ESBJERG.

Department of Ecology and Molecular Biology, The Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark.

Summary.

A crude destruxin extract was used to examine the activity against caterpillars of turnip moth, *Agrotis segetum*, and the large white, *Pieris brassicae*. Toxins were spread over the surface of the diet up to a concentration of 834 µg/cm^2. *P. brassicae* proved to be the far most susceptible: the highest destruxin concentration caused a 100% mortality within 5 days but only within 11 days treated *A. segetum* died. However, destruxin treated diet caused a reduced food intake among *A. segetum* larvae, so we suggest that *A. segetum* larvae died of starvation due to a repellant effect rather than by a toxification.

Introduction.

Destruxins are secondary metabolites from *Metarhizium anisopliae* and are believed to play a role in the fungal pathogenesis (Roberts, 1981). Their mode of action has still to be defined. The destruxins alone have shown to be toxic against insects from a wide range of orders. Among Diptera, destruxins are reported to be toxic to larvae of the mosquito, *Culex pipiens*, as well as adults of fruit flies, *Drosophila melanogaster*, common house flies, *Musca domestica*, and onion maggots, *Delia antiqua* (Poprawski *et al.*, 1985; Vey *et al.*, 1987). *Per os* experiments with Lepidopteran larvae demonstrated toxicity to the greater wax moth, *Galleria mellonella* and the silkworm, *Bombyx mori* (Roberts, 1981; Vey & Quiot, 1989). Also Coleopteran larvae, as *Cetonia aurata* and *Oryctes rhinoceros*, have been reported to be susceptible to destruxins (Fargues *et al.*, 1985).

In a project concerning the possibility to use destruxins as new biopesticides, we have initiated bioassays of toxicity of destruxins to larvae from two lepidopteran species. The turnip moth, *Agrotis segetum* (cutworms), and the large white, *Pieris brassicae*, inhabiting very different ecological niches.

Cutworms feed on most root crops and may cause serious damage under population outbreaks. All larval stages stay mostly in the upper soil layer around the roots, but 1st and 2nd instars move to the foliage to feed (Esbjerg, 1992). Due to their cryptic behaviour cutworms are difficult to hit by spraying. *P. brassicae* larvae appear in colonies on leaves of brassica plants, which they can defoliate completely. Increasing problems with this pest insect have occured in Denmark during the last years. In conventional farming major control measures are taken by chemicals, but on limited areas products based on *Bacillus thuringiensis* are used.

Results from the initial bioassays against these two insect pests are presented below.

Materials and methods.

Destruxins: Destruxin was provided by Alain Vey, INRA, St. Christol, Les-Alès, France, as a crude extract isolated from *Metarhizium anisopliae* (Ma23).

Insects: *Agrotis segetum* were selected from a laboratory culture fed with artificial diet (Hansen & Zethner, 1979). The culture had been reinitiated with field collected larvae during autumn 1994.

Pieris brassicae larvae were obtained from eggs send regularly by Paul Jarret, Horticultural Research International, Littlehampton, UK. They were provided an artificial diet (David & Gardiner, 1965) as soon as the eggs hatched.

Bioassays: Destruxins were dissolved in ethanol/acetone (1:1) [highest concentration] and the following concentrations were obtained by dilution with water. Application of water and solvents (ethanol/acetone 1:1) were added as controls. The toxin was spread on the surface of the diet, resulting in the following concentrations: 834, 167, 33, 6.7, and 1.3 µg destruxin/cm^2, respectively. Solvents were allowed to evaporate for 1 hour in a fume hood.

A. segetum larvae were kept individually during bioassays to prevent cannibalism. Plastic frames at equal size subdivided into 9 small rooms made up 1 cage with 9 larvae. A treatment where the larvae were provided only moist filter paper (eg. without food) was added. Two cages with 1st instar cutworms were used pr. treatment (= 18 larvae) (5 replicates). Larvae in each treatment were weighed together to ensure an equal mean larval size (0.83 mg/larvae) in different treatments and replications. Cages were placed at 20°C at a 16 h photoperiod. Once a week (for four weeks) the larvae were provided new, toxin treated food. The mortality was recorded daily for 3 weeks. After 4 weeks the remaining larvae were provided untreated fodder until pupation. For one replication the time until pupation and pupal weight was also recorded.

Tests with *Pieris brassicae* larvae were performed in small petridishes (9 cm^2) with the artificial diet. 15 larvae (3rd instar) were transferred to each petridish (2 petridishes/ treatment = 30 caterpillars) (3 replicates). Larvae were incubated as *A. segetum* but mortality was recorded daily for only 6 days.

Results.

For both insect species mortalities of 100% were obtained using the highest toxin concentrations.

As seen from figure 1, *Pieris brassicae* was very susceptible to destruxins. The highest concentration (834 µg/cm²) resulted in 100% mortality within 5 days, and even the concentration of 6.7 µg/cm² caused a mortality of more than 80% within the 6 days.

Agrotis segetum was much less susceptible (fig. 2) to destruxins. Solely the three highest doses resulted in mortalities different from the controls. After 11 days only the highest dose (834 µg/cm²) caused 100% mortality. Mortalities observed for the two highest concentrations did not differ from the treatment where larvae were held on moist filter paper (starved).

For all destruxin concentrations, even the ones that gave no mortality, a reduction of larval feeding was observed and the higher the destruxin concentration, the higher effect of the reduced food intake. Larvae exposed to the highest destruxin concentrations almost completely stopped feeding, which could be seen by the lack of frass in the cages. However, the resulting pupae from the replicate exposed to the toxins for 4 weeks did not show any clear differences in weight, between the control and the treatments (data not shown). Only the time of pupation of larvae subjected to 6.7 µg/cm² was delayed. This delay was about 1 week (fig. 3), compared with the control and the lowest destruxin concentration at 1.3 µg/cm², the same delay as was suggested by the weight of the larvae at the end of the destruxin treatment.

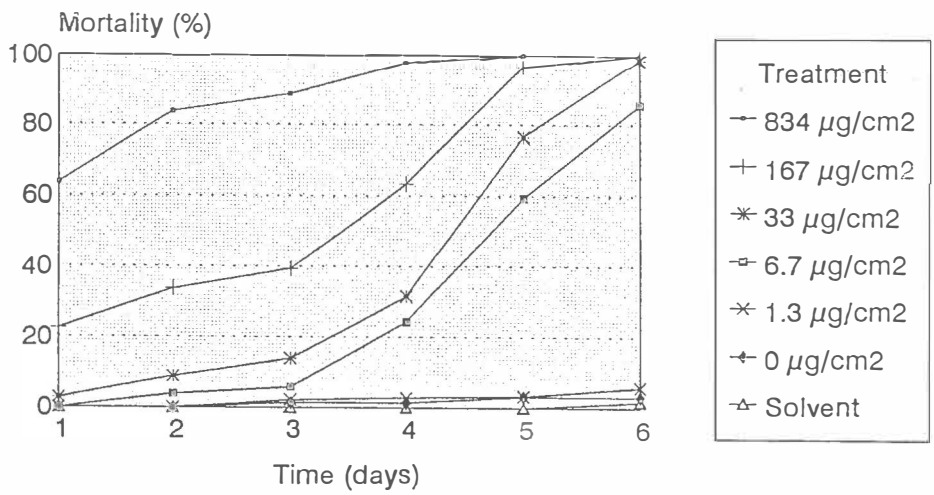


Figure 1. Mortality of 3rd instar *Pieris brassicae* larvae subjected to different destruxin concentrations from beginning of exposure.
Means of 3 replicates, except 'solvent' (2 replicates). 30 larvae/treatment/replicate.

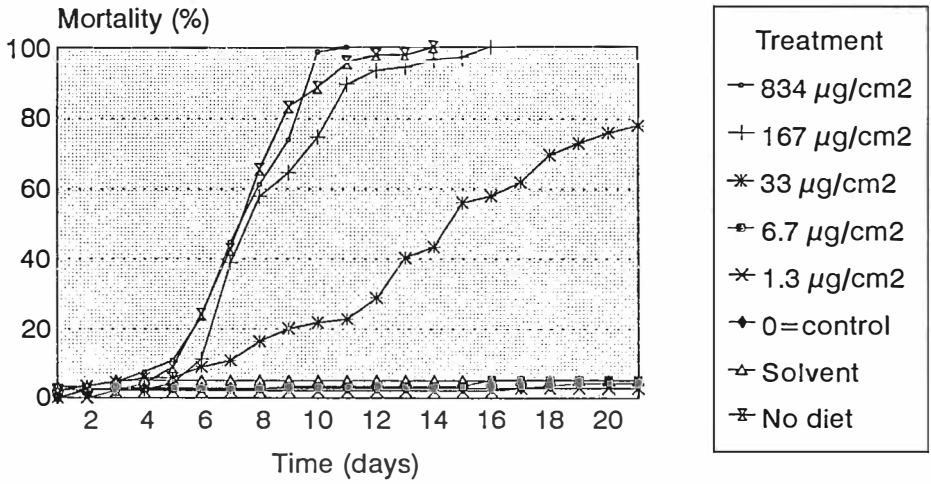


Figure 2. Mortality of 1st instar *Agrotis segetum* larvae subjected to different destruxin concentrations from beginning of exposure.
Once a week larvae were provided new toxin treated diet. Means of 5 replicates, except 'solvent' (2 replicates) and 'no diet' (4 replicates). 18 larvae/treatment/replicate.

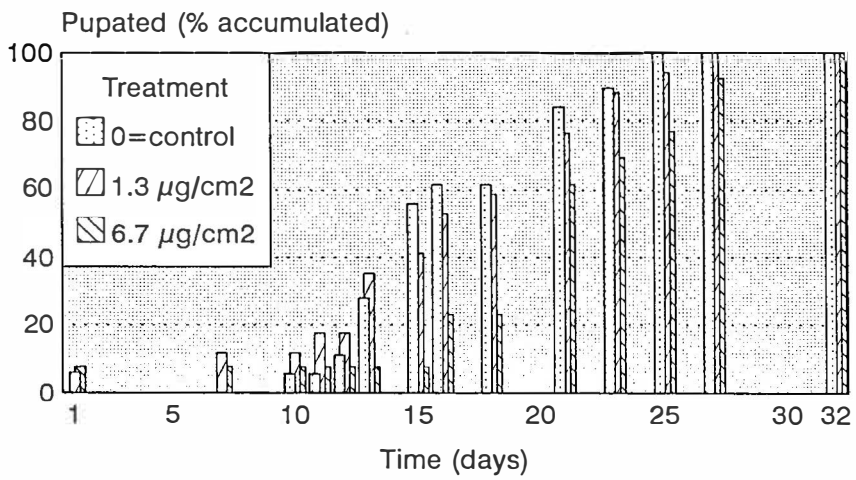


Figure 3. Accumulated percentage of pupated *Agrotis segetum* larvae after treatment with destruxins.
1st instar larvae, approximately 1 week old, were provided destruxin treated food for 4 weeks, and thereafter untreated diet until pupation. Day 1 refers to the first observation of a pupa.

Discussion.

Both insect species were susceptible to destruxins in a dose dependent manner, but *Agrotis segetum* was much less susceptible to destruxins than *Pieris brassicae*. The suppression of food intake was probably due to a repellant effect of destruxins and not to a damage of the digestive tract, since larvae subjected to the lower destruxin doses started to feed on the filter paper instead of the diet. The long time needed to kill *A. segetum* larvae together with their reduced feeding, and the results obtained when larvae were starved, suggest that *A. segetum* died of starvation due to strong feeding repellence rather than due to toxic effects. A repellant effect was also seen when house flies (*Musca domestica*) were exposed to destruxins through their diet (Thomsen & Eilenberg, 1995). When these flies had the choice, they clearly preferred untreated food to destruxin treated.

Although lower destruxin concentrations had a minor feeding deterrent effect on *A. segetum* larvae, they pupated normally but apparently with a small delay compared to the control. This also suggests that there is no cumulative effect of a continued intake of low doses of destruxins by *A. segetum*. They are possibly able to detoxify the destruxins into harmless compounds. This phenomenon has already been observed with a Coleoptera larva, *C. aurata*, (Fargues *et al.*, 1985), but the route of detoxifation is still unknown.

References.

DAVID, W. A. L.; GARDINER, B. O. C. 1965. Rearing *Pieris brassicae* L. larvae on a semisynthetic diet. Nature (London) **207**:882-883.

ESBJERG, P., 1992. Temperature and soil moisture - two major factors affecting *Agrotis segetum* Schiff. (Lep., Noctuidae) populations and their damage. IOBC/WPRS Bulletin **15**:82-91.

FARGUES, J.; ROBERT, P.-H.; VEY, A. (1985). Effet des destruxines A, B et E dans la pathogénèse de *Metarhizium anisopliae* chez les larves de Coléoptères *Scarabaeidae*. Entomophaga **30**:353-364.

HANSEN, L. Ø.; ZETHNER, O., 1979. Techniques for rearing 26 species of Noctuidae (Lepidoptera) on an artificial diet. Yearbook, Royal Vet.- and Agric. University, Copenhagen, Denmark. pp. 84-97.

POPRAWSKI, T. J.; ROBERT, P.-H.; MANIANIA, N. K. (1985). Susceptibility of the onion maggot, *Delia antiqua* (Diptera: Anthomyiidae), to the mycotoxin destruxin E. The Canadian Entomologist **117**:801-802.

ROBERTS, D. W., 1981. In: BURGES, H. D. (ed). Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, London, UK. pp. 441-464.

THOMSEN, L.; EILENBERG, J. 1995. Future possibilities of using insect pathogens to control pest insects in vegetables with special emphasis on fungal metabolites. NJF-reports (in press).

VEY, A.; QUIOT, J.-M., 1989. Effet cytotoxique *in vitro* et chez l'insecte hôte des destruxines, toxines cyclodepsipeptidique produites par les champignon entomopathogène *Metarhizium anisopliae*. Canadian Journal of Microbiology **35**:1000-1008.

VEY, A.; QUIOT, J.-M,; VAGO, C., 1987. Mode d'action insecticide d'une mycotoxine, la destruxine E, sur les diptères vecteurs et disséminateurs de germes. Comptés Rendus de l'Academie de Science, Paris, Serie III. **304**:229-234.

SECONDARY METABOLITES PRODUCED BY ENTOMOPATHOGENIC FUNGI OF THE GENERA *ZOOPHTHORA* AND *PAECILOMYCES*

A. ZABŻA[1], J. PIĄTKOWSKI[2], B. GREB-MARKIEWICZ[1] AND J. BUJAK[1]

(1) Institute of Organic Chemistry, Biochemistry and Biotechnology, Technical University of Wrocław, Wyb. Wyspiańskiego 27, 50-370 Wrocław, Poland

(2) Institute of Microbiology, University of Wrocław, S. Przybyszewskiego 63/77, 51-148 Wrocław, Poland

INTRODUCTION

Entomopathogenous fungi have been found to be effective against several pests of crops (PICKET *et al.* 1990, WILDING *et al.* 1983). Therefore, the chemical analysis of their secondary metabolites, which could be engaged in intoxication of insect's organism, is very important from practical point of view. In this paper we present the results of our studies on the influence of pH and Zn^{++}, Co^{++}, Mg^{++}, Cd^{++} and SeO_3^{--} ions on the formation of some metabolites by two entomopathogenic fungi - *Zoophthora neoaphidis* and *Paecilomyces sp.*. Preliminary chemical analysis (HPLC) and antimicrobial properties are described.

MATERIALS AND METHODS

Microorganisms

Source: The strain of *Paecilomyces* sp. was obtained from Collection of the Research Centre for Agricultural and Forest Environmental Studies of the Polish Academy of Science in Poznań, (cat. nr 2428c). The strain of *Z. neoaphidis* was received from Collection of the Agricultural University, Department of Entomology, Szczecin (cat. nr 13D).

Bacteria species for biological tests: *Staphylococcus aureus* (PCM 2054) and *Enterococcus phaecalis* (PCM 1861) were provided from the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wrocław, and *Pseudomonas cepacia* (PZ 2), *Enterobacter aglomerans* (PZ 1) and *Escherichia coli* (781) from the Institute of Microbiology, University of Wroclaw.

Cultivation of microorganisms: The fungi were maintained on nutrient medium recommended by MÜLLER-KÖGLER (1941).

Analytical and preparative HPLC chromatographies were performed on the HPLC Beckman apparatus (Model 420) (column Ultrasphere-octyl; 10x250 mm; injection-20µl eluent: MeOH/H_2O/acetonitryl/tetrahydrofuran 80:20:15:5; flow rate 1.2ml/min), with Hewlett-Packard recorder (Model HP 3396): (detection UV at λ = 254 nm).

Stimulation of the metabolites secretion

After incubation (2-3 weeks) on solid medium (in Petri dishes, 10 cm diameter) consisted of: bacto pepton (12g/l), yeast extract (5g/l), casein hydrolizate (2g/l), glucose (20g/l), bacto agar (20g/l), with additional amounts of: 10, 50, or 100 µg/ml of $ZnSO_4x7H_2O$, or $MgSO_4x7H_2O$, or $CdSO_4x8H_2O$, or $CoCl_2x6H_2O$, or 1, 5, 10 µg/ml H_2SeO_3) at pH = 4.8 or pH = 8.0, (stabilised

by Sörensen phosphate buffer) at **28°** C (Stage I), the mycelia were transferred on the analogical media at pH = 8.0 or 4.8, but at **36°** C and incubated for next 3 days (Stage II) (Table 1). In the second stage of cultivation on the surface of mycelium yellow (or dark yellow) drops appeared. Studies were limited only to metabolites contained in these drops.

Determination of antibacterial activity

Antibacterial activity was examined with metabolites produced by *Z. neoaphidis*, only. Five species of bacteria were added separately to the 100 µl samples of secreted drops and nutritional broth as a control. The viability of the bacteria was measured in "colony forming units" [c.f.u.] after 2 and 4 hours of incubation at 36°C (Table 2).

RESULTS AND DISCUSSION

The drops of metabolites appeared on surface of mycelia when they reached the size about 5 cm in diameter. The influence of ions and pH on their production, and antibacterial activities were presented in Table 1 and 2 and Fig. 1.

Table 1
Influence of ions and pH on the secretion of the metabolites by *Z. neoaphidis*

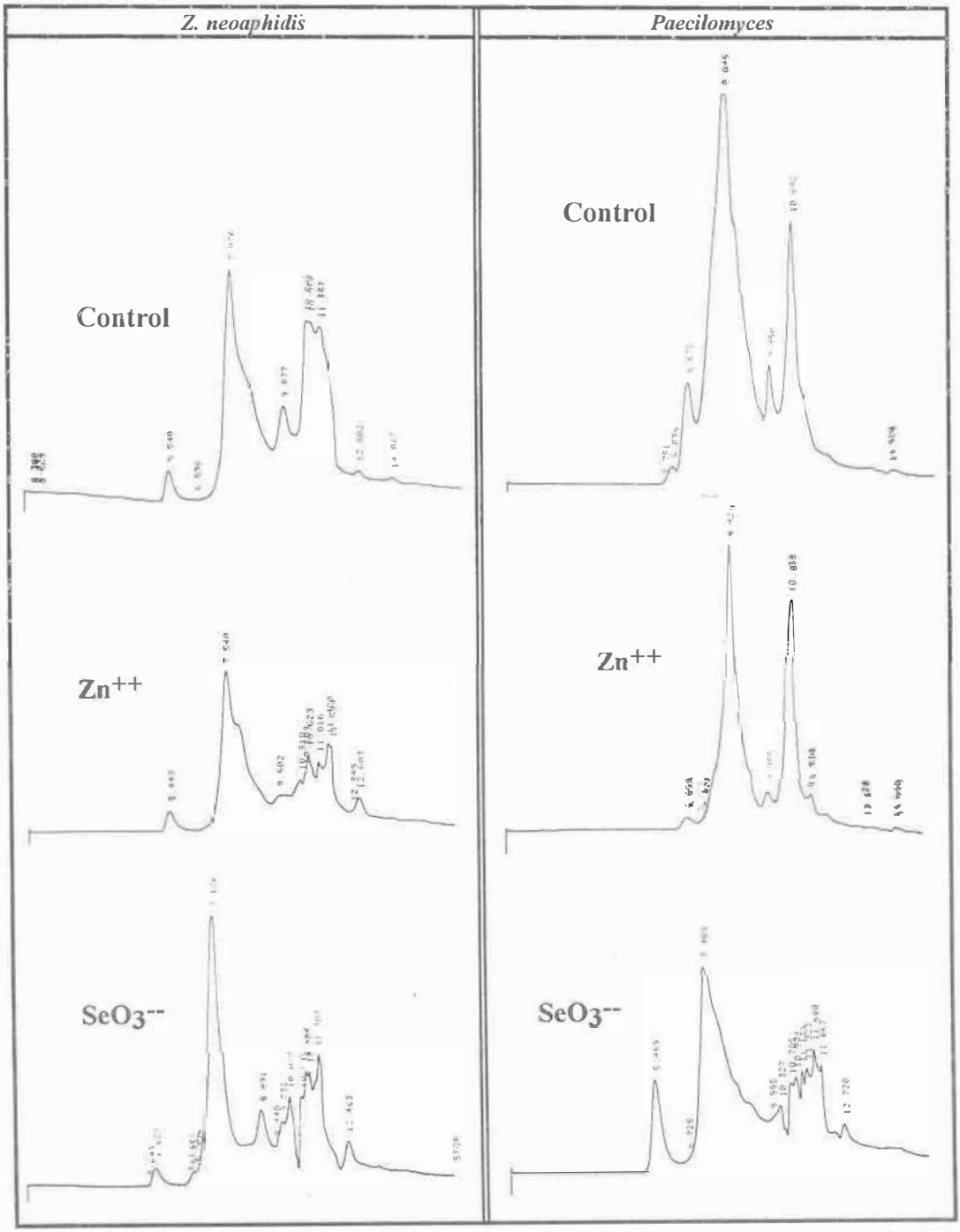
Stage I		Stage II		
		Secretion at pH		Size of mycelium
ions	pH	4.8	8	[cm in diameter]
Co^{++}, 100 µg/ml	4.8	–	++	5.5
	8.0	–	+-	4.8
Cd^{++}, 10 µg/ml	4.8	–	++	5.5
	8.0	–	+	4.3
Mg^{++}, 100 µg/ml	4.8	–	++	5.5
	8.0	–	-	4.7
Zn^{++}, 100µg/ml	4.8	–	+++	5.2
	8.0	–	-	3
SeO_3^{--}, 1 µg/ml	4.8	–	+	5
	8.0	–	+	4.8
Control	4.8	–	+	5.4
	8.0	–	+-	4.7

*/Stage I: incubation on the solid medium (2-3 weeks at 28° C).
**/ Stage II: mycelia were transferred on the new solid medium and incubated for the next 3 days at 36° C.
"+++" ~40µl ,"++" ~10 µl, "+" 3 µl of liquid secreted on ~ 5 cm^2 of mycelium surface.

Weak acidity of medium (pH = 4.8) was needed to induce formation of drops on the surface of mycelium. The higher stimulating effects were observed when SeO_3^{--}, Cd^{++} or Zn^{++} ions were applied. Smaller, but clearly visible stimulation was also noticed in the cases of the other ions tested.

Figure 1

Chromatograms of metabolites produced by *Paecilomyces* and *Z. neoaphidis* in presence of Zn^{++} and SeO_3^{--} ions



The metabolites produced by analysed fungi presented rather a complicated mixture of compounds (HPLC chromatograms, Fig. 1). As concerned diversity of products, all tested ions seemed to exert similar effects. The HPLC chromatograms of metabolites produced in the presence of Co^{++} and Cd^{++} ions were very similar to that observed for Mg^{++} ion.

On the HPLC chromatograms, the main amounts of metabolites were observed at retention time [Rt] 7.1 - 7.9 and 10.5 - 11.5 for both microorganisms tested. Smaller amounts of metabolites with Rt ~ 5.5 and 9.5 were also detected. The SeO_3^{--} and Zn^{++} ions exerted the most visible effects on metabolites production. The influence of Co^{++}, Cd^{++} and Mg^{++} ions was weaker but clearly visible.

In the case of *Paecilomyces*, Zn^{++}, Cd^{++} and Mg^{++} ions stimulated production of metabolites with Rt ~10.8. SeO_3^{--} and Co^{++} ions clearly diversified structures of products with Rt = 9.8-12 range. Isolations and structure elucidation of pure compounds are in progress.

The results of antibacterial activities of the metabolites analysed presented in Table 2 indicate that all bacteria tested were affected by metabolites produced by *Z. neoaphidis*. The highest effects were observed for *Enterococcus phaecalis* and *Staphylococcus aureus*, both microorganisms were killed during two hours.

Table 2
Influence of secreted metabolites of *Z. neoaphidis* on the growth and viability of selected species of bacteria

Bacteria		number of c.f.u.* after time (h)		
		0	2	4
Escherichia coli	control	$242x10^3$	$271x10^4$	$87x10^5$
	with metabolites	$242x10^3$	$97x10^4$	$34x10^5$
Pseudomonas cepacia	control	$70x10^3$	$55x10^4$	$63x10^5$
	with metabolites	$70x10^3$	$55x10^4$	$63x10^5$
Staphylococcus aureus	control	$95x10^3$	$74x10^4$	$95x10^5$
	with metabolites	$95x10^3$	0	0
Enterococcus phaecalis	control	$130x10^3$	$38x10^4$	$35x10^5$
	with metabolites	$130x10^3$	0	0
Enterobacter aglomerans	control	$96x10^3$	$152x10^4$	$90x10^5$
	with metabolites	$96x10^3$	$98x10^4$	$56x10^5$

* c.f.u. = colony forming units

REFERENCES

MÜLLER-KÖGLER , E., 1941. Zur Isolierung und Kultur Insektenpathogener Entomophthoraceen. Z. Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz, **51**, 124-274.

PICKET, J.A., WADHAMS, L.J. AND WOODCOCK, C.M. 1990. New approaches to the develompent of semiochemicala for insect control. Proc. Conf. Insect Chem. Ecol., Tabor, 339-345.

WILDING, N., BROBYN, J.P. AND MARDELL, S.K. 1983. Tothamsted Experimental Station Raport for 1983, Part 1, 95.

Acknowledgements: The research was sponsored by the State Committee for Scientific Research Republic of Poland (Grant 6 PO4B 001 09).

A comparison between MYCOTAL (*Verticillium lecanii*) and *Aschersonia aleyrodis* for the control of whitefly

R.K. van der Pas, W.J. Ravensberg, A.C. den Braver, A.C. van Buijsen and M. Malais

Koppert B.V., Veilingweg 17, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands

Summary

The control of whitefly with two insect-pathogenic fungi, *Verticillium lecanii* (MYCOTAL) and *Aschersonia aleyrodis*, was compared under laboratory conditions at different relative humidities (RH) as well as in tomato and cucumber crops in commercial glasshouses in the Netherlands. For the experiments conidiospores of *V. lecanii* were formulated as a wettable powder (MYCOTAL) and pycnidiospores of *A. aleyrodis* were formulated as water dispersable granules. The mortality of whitefly after one treatment with *V. lecanii* in detached leaf bio-assays at 60, 75 and 85% RH was 45, 55 and 75% and 42, 45 and 30% for *A. aleyrodis*. External growth of *V. lecanii* mycelium and sporulation was not visible at a RH of 60% and 75%, at 85% RH only 25% of the whitefly larvae showed external sporulation. For *A. aleyrodis* visible external sporulation was 25, 28 and 30% at 60, 75 and 85% RH respectively.
In the glasshouse trials both fungi reduced whitefly populations by approx. 80%-90% in both cucumber and tomato. *V. lecanii* gave a quicker knock-down effect than *A. aleyrodis*, although an effect was earlier visible when using *A. aleyrodis*.

1. Introduction

V. lecanii is a well-known pathogen of arthropods: it was first described in 1861 and has been collected from numerous species of insects, spiders and mites (Rombach et al., 1988). It is natural occurring in temporate and subtropical zones. Since the end of the last century, it has been tried to develop a microbiological insecticide against several insects based on this fungus. Between 1980 and 1985 a microbial insecticide, MYCOTAL, based on a specific strain of *V. lecanii*, was developed against whitefly (*Trialeurodes vaporarorium*) in the UK (Hall, 1982). Although the product did not work sufficiently enough in trials, Koppert resumed the product in 1988. Improvement of the production and formulation resulted into a wettable powder based on the conidiospores of this strain. MYCOTAL is commercially sold in the Netherlands, United Kingdom, Switzerland, Denmark and Sweden as a product against whitefly with a side effect on thrips. The fungus infects the insects through their external cuticle by, at first, adhesion and germination of the spores on the cuticle followed by penetration into the body cavity where it infects and destroys the tissues (Samson et al., 1988).
The activity of MYCOTAL is largely dependent on the RH (Quinlan et al., 1988). At high RH it can control whitefly very good, but with decreasing RH the activity of MYCOTAL is proportionally declining.

Also the effect of MYCOTAL is difficult to observe, infected larvae/pupae colour white and only at a high RH visible external fungal growth is shown. Since most of the time the RH in a glasshouse is below 80% there is a need to find a fungus that is less dependent on a high RH. A possible candidate is *A. aleyrodis*, a fungus which originates from more tropical and drier zones. It is a well-known and described pathogen of scales and whiteflies. Infected larvae/pupae colour orange even at a low RH. The mode of action is comparable to that of *V. lecanii*. The control of whitefly with both fungi was compared under laboratory conditions at different RH as well as in trials in commercial tomato and cucumber crops in glasshouses in the Netherlands.

2. Material and Methodes

Products

MYCOTAL is a wettable powder based on the spores of a specific strain of *V. lecanii* (KV01***). The product contains 10^{10} spores/gram and the spray solution 10^7 spores/ml.
The pycnidiospores of *A. aleyrodis* (CBS 334.87) were formulated as water dispersable granules containing 10^{10} spores/gram and $5x10^6$ spores/ml spray solution.

Bio-assay

To test efficacy of both fungal products at different RH's, a detached leaf bio-assay was used. Leaf discs, supported on 1% water agar, were put in a round "petri dish-type" tray with a lid (ϕ 77 mm; height 31 mm; Bock, Art.Nr.41113). In order to allow sufficient gaseous exchange four holes were made in the side of the tray (ϕ 1 cm) and one in the lid (ϕ 6.5 cm) covered with nylon mesh.
Treatments were applied once with a Potter spray tower, 3.5 ml per tray. In order to obtain the desired RH trays were incubated above a saturated salt solution of KCl (≈85% RH), NaCl (≈75% RH) and $Ca(NO_3)_2$ (≈55% RH). Incubation conditions were 22°C, 16h light. Assessments were carried out after 10 days (MYCOTAL) and 14 days (*A. aleyrodis*).

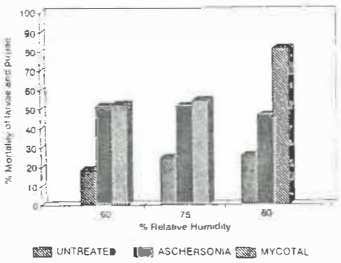
Field trials

The efficacy of both products was tested in cucumber and tomato crops in commercial glasshouses in the Netherlands. Both products were sprayed three times at weekly intervals with standard spraying equipment at a rate of 3 kg per hectare (MYCOTAL) or 1.5 kg per hectare (*A.aleyrodis*) in 1500-2000 litres of water. Spraying was always done at the end of the day. Treated areas ranged from 500-900 m^2. An untreated control area was incorporated in each glasshouse. Normal operating procedures were maintained, no measures were taken to increase the humidity. The average RH during the trials was 70-80% in both crops. In the treated area 2 to 4 sampling plots and in the untreated control area 1 to 4 sampling plots were inserted depending on the pest situation in the glasshouses. Sampling plots ranged from 40 to 60 m^2. The crops were sprayed in week 0, 1 and 2 after the start of the experiments. The first counting was performed just before the first spraying.
To determine the percentage mortality of whitefly caused by the fungi, 25 leaves were picked weekly from the sampling plots. Countings were made of numbers of dead (not evidently infected), infected, and living instars of *T. vaporariorum*, using a binocular microscope.

3. Results

Bio-assays

At 60% and 75% RH there was no difference in mortality of larvae and pupae of *T.vaporariorum* treated with MYCOTAL or *A. aleyrodis* . Only at 85% RH there was a significant difference in mortality (see fig. 1). Although MYCOTAL performed similar as or even better than *A. aleyrodis* in the bio-assays, it was difficult to actually see the effect of the treatment. With *A. aleyrodis* this was very easy because the infected larvae and pupae coloured orange, even at low a RH (see fig. 2).



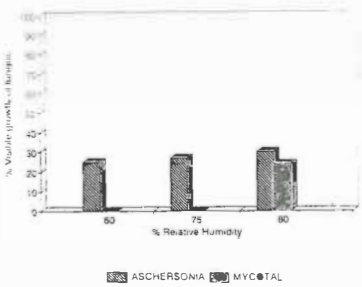


Fig. 1 (left) Control of *T. vaporariorum* at different RH's in detached leaf bio-assays with MYCOTAL and *A. aleyrodis*

Fig. 2 (right) Visible external growth of *V. lecanii* and *A. aleyrodis* on larvae and pupae of *T. vaporariorum* in detached leaf bio-assays.

Field trials

Both fungi reduced whitefly populations by approx. 80%-90% in both crops. In cucumber this was reached after 2 weeks (fig. 3 and 4), in tomato it took longer to reach this mortality (fig. 5). *V. lecanii* showed a quicker knock-down effect than *A. aleyrodis*, although an effect is earlier visible when using *A. aleyrodis*.

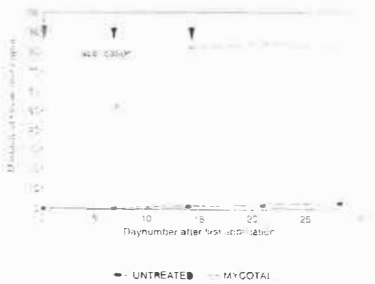
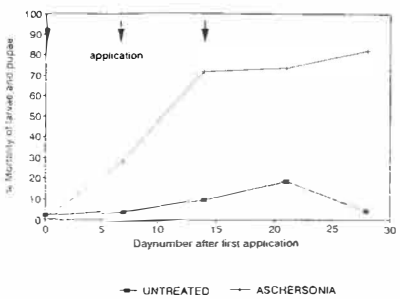


Fig. 3 Control of *Trialeurodes vaporariorum* in cucumber with MYCOTAL



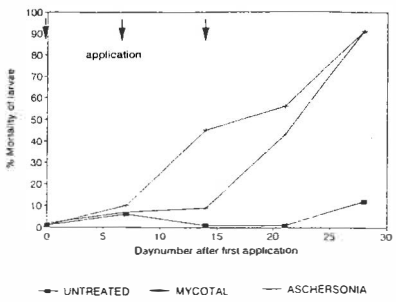


Fig. 4 (left) Control of *Trialeurodes vaporariorum* in cucumber with *Aschersonia aleyrodis*

Fig. 5 (right) Control of *Trialeurodes vaporariorum* in tomato with MYCOTAL and *Aschersonia aleyrodis*

4. Discussion

The RH at which the spores germinate is much higher than the overall glasshouse humidity. It is obvious that not the RH in the glasshouses or bio-assay trays is important for a good control, but the microclimate in the phyllosphere. The RH at the underside of a leaf can be high enough for good control even when the overall humidity in the glasshouse is low (Ravensberg et al., 1990). That is why the control of whitefly with MYCOTAL was comparable with, or even better than, the control with *A. aleyrodis*, even at low overall RH. Compared with *A. aleyrodis* MYCOTAL even demonstated a quicker knock-down. Although *A. aleyrodis* shows more visable external growth and therefore is much easier to observe for growers, this is not enough reason to commercialize (this strain) *A. aleyrodis*. Not only because the efficacy is not significantly better than that of MYCOTAL, also the production costs are much higher. Still research is being continued to find or select a better strain of *A. aleyrodis* or *V. lecanii* for the control of whitefly.

5. References

HALL, R.A., 1982. Control of whitefly *Trialeurodes vaporariorum* and cotton aphid *Aphis gossypii* in glasshouses by two isolates of the fungus *Verticillium lecanii*. Annals of Applied Biology, 101: 1-12.

QUINLAN, R.J., 1988. Use of fungi to control insects in glasshouses. In: Fungi in biological control systems, ed. M.N. Burges: 19-36. Manchester University Press, Manchester.

RAVENSBERG, W.J., MALAIS M. & VAN DER SCHAAF, D.A., 1990. Applications of *Verticillium lecanii* in tomatoes and cucumbers to control whitefly and thrips. SROP/WPRS Bull. XIII/5: 173-178.

ROMBACH, M.C. & GILLESPIE, A.T., 1988. Entomogenous hypomycetes for insect and mite control on greenhouse crops. Biocontrol news and information 9: 7-18.

SAMSON, R.A., EVANS, H.C. & LARGE, J., 1988. Atlas of Entomopathogenic fungi: 128-139. Wetenschappelijke uitgeverij Bunge, Utrecht

FUNGI OCCURRING ON SUGARBEET NEMATODE (*HETERODERA SCHACHTII* SCHMIDT) IN WIELKOPOLSKA REGION

D. Sosnowska
Department of Biological Methods of Pest Control and Quarantine, Institute of Plant Protection, Miczurina 20, 60-318 Poznań, Poland

Summary

Soil samples from two sugar beet fields in Wielkopolska region were examined to identify microorganisms colonizing cysts of sugarbeet nematode (*Heterodera schachtii* Schmidt). Over eight hundred fourteen surface sterilized cysts were analyzed in the laboratory. The most frequently occurring pathogens were: *Alternaria spp.*, *Fusarium sambucinum*, *Cylindrocarpon spp.* (close to *C.destructans*), *Scopulariopsis spp.*, and *Verticillium chlamydosporium*. Overall parasitization level of examined cysts increased from 16% during the winter time to 50% during the spring. *Cylindrocarpon spp.* was the predominant species.

C.destructans and *V.chlamydosporium* are considered as pathogens of cysts of *H.schachtii* and may have the potential for biological control of nematodes.

Introduction

The beet cyst nematode (*Heterodera schachtii* Schmidt) is of particular economic importance as it causes severe yield losses throughout the temperate zones of the world. In Poland 1/3 of beet fields is infested by this pest causing 50% of yield sugar and 40% of yield roods reductions (Boczek, 1988).

Control of nematode by chemicals, crop rotation, resistant cultivars and management practices is impracticable, and recently, biological control with nematophagous fungi belongs to alternative methods of control this pest.

This paper reports a survey of beet fields in Wielkopolska region in Poland, to examine the type of fungi associated with *H.schachtii* as a preliminary to identifying those which influence nematode populations.

Material and Methods.

Soil samples were collected during January-March from Poznań field in 1992 (661 cysts) and in April 1994 from beet field in Kędzierzyn (near Niechanowo) (153 cysts). A

modification of Kerry's method (1977) was used. Cysts were washed 5 times in sterile distilled water. Than transferred to sterile distilled water containing 0,05% streptomycin sulfate. After the suspensions were agitated, a samples was pipetted onto plates containing 0,01% streptomycin sulfate and were incubated at 21°C for 24-48 hr. Cysts were examined at random for growth of fungal hyphae under a microscope. To aid in the identification of fungal parasites, some cysts were transferred to Petri plates containing glucose-agar to allow fungi to grow and sporulate.

Results and Discussion.

A total of six isolates of fungi was recovered from the 814 cysts of *H.schachtii* (Table 1).

Table 1. Fungi isolated from cysts and eggs of *Heterodera schachtii* (Wielkopolska region).

Eggs	Cysts
Cylindrocarpon spp.	Alternaria spp.
Fusarium sambucinum Fuck.	Cylindrocarpon spp. (close to C.destructans)
Verticillium chlamydosporium Godd.	Fusarium sambucinum Fuck
	Fusarium spp.
	Scopulariopsis spp.

Overall parasitization level of examined cysts increased from 16% to 36% during the winter time and from 40% to 50% during the spring (Table 2).

Cylindrocarpon spp. was the predominant species on eggs and cysts. Infected eggs were reddish brown due to the content of fungus hyphae. This species which infested about 50% of cysts, is already reported as significant factor reducing *H.schachtii* populations on the plots studied in West of Poland region (Banaszek et all., 1990).

V.chlamydosporium was isolated from eggs of *H.schachtii*. Infected eggs were characteristically yellow-brown and the content, permeated by hyphae, was often slightly shrunken, sometimes causing convolution of the egg wall. *V.chlamydosporium* occurred on *H.schachtii* eggs in many countries and was considered as pathogen of cysts and eggs, and may have the potential for biological control of nematodes (Bursnall, Tribe, 1974; Tribe, 1979; Crump & Irving, 1992). This species has not been reported from

H.schachtii in Wielkopolska region before (Banaszek et all., 1990). *V.chlamydosporium* was frequently isolated from eggs and cysts from soil, when analyzed shortly after sampling. With longer storage its frequency appeared to decrease.

Table 2. Percent of parasitization of eggs and cysts of *Heterodera schachtii* in different fields.

	% of parasitization	
Number of cysts	cysts	eggs
1992 - Poznań (January)		
20	25	-
45	35,5	-
34	26	-
51	16	-
Mean: 37,5	25,6	-
1992 - Poznań (March)		
86	50	40
74	40	50
61	50	30
290	50	40
Mean: 127,8	47,5	40
1994 - Kędzierzyn (April)		
39	45	20
63	20	30
27	50	30
24	38	24
Mean: 38,3	38,3	26

Saprophytic fungi like *Fusarium spp.* and *Scopulariopsis spp.* may play important role in nematode population. They have a big chitin- and proteinactivity and cysts wall can be an important food source for them. They can also produce a mycotoxines, and due to we often observed dead and deformed larvae inside cysts covered with mycelium of this species. This observations confirmed Wronkowska, Janowicz (1988) experiments and conclusions.

Much work is required to understand the importance of fungal attack on cyst nematode population dynamics.

Conclusions.

1. The soil roots of beet in Wielkopolska region contain a diverse group of fungi which are intimately associated with cysts of *H.schschtii*. Some of these fungi may play a regulatory role in the population dynamics of the nematode species and warrant further investigation.
2. A total of six isolates of fungi was recovered from the cysts and eggs of *H.schachtii*.
3. Overall parasitization level of examined cysts increased from 16% during the winter time to 50% during the spring.
4. *Cylindrocarpon* spp. was the predominant species. *Verticillium chlamydosporium* was isolated from eggs and was not reported in Wielkopolska region before.

Acknowledgments

The assistance of Prof. S.Balazy in identification of some of the fungi, and Dr. S.Wolny in supplying of cysts samples is gratefully acknowledged.

References.

BANASZEK, H., WRONKOWSKA, H., CYRANOWICZ, H., KOBA, M. 1990. Grzyby wystepujace w cystach matwika burakowego podczas uprawy buraka cukrowego w dwuletnich rotacjach. Zeszyty problemowe Postepow Nauk Rolniczych, No 391: 15-23.

BOCZEK, J. 1988. Nauka o szkodnikach roslin uprawnych. Warszawa: 338 pp.

BURSNALL, L.A., TRIBE, H.T. 1974. Fungal parasites in cysts of *Heterodera*. II. Eggs parasites of *H.schachtii*. Trans. Br. mycol. Soc. 62 (3): 595-601.

CRUMP, D.H., IRVING, F. 1992. Selection of isolates and methods of culturing *Verticillium chlamydosporium* and its efficacy as a biological control agent of beet and potato cyst nematodes. Nematologica 38: 367-374.

KERRY, B.R., CRUMP, D.H. 1977. Observations of fungal parasites of females and eggs of the cereal cyst nematode, *Heterodera avenae* Woll. and other cyst nematodes. Nematologica 23: 193-201.

TRIBE, H.T. 1979. Extent of disease in population of *Heterodera*, with special reference to *H.schachtii*. Annals of Applied Biology 92: 61-72.

WRONKOWSKA, H., JANOWICZ, K. 1988. Wplyw grzybow wyizolowanych z cyst matwika ziemniaczanego (*Globodera rostochiensis* Woll.) na tego nicienia w badaniach in vitro. Zeszyty Naukowe Akademii Rolniczo-Technicznej w Olsztynie, No 47: 92-99.

ENTOPATHOGENIC FUNGI INHABITING FOREST LITTER AND SOIL IN PINE FORESTS WITH DIFFERENT LEVELS OF ENVIRONMENTAL POLLUTION

C. Bajan, K. Kmitowa, E. Mierzejewska, E. Popowska-Nowak[1], R. Miętkiewski, R. Górski, Z. Miętkiewska[2]

(1) Institute of Ecology Polish Academy of Sciences, Dziekanów Leśny, 05-092 Łomianki, Poland
(2) Agricultural and Teachers University, Department of Plant Protection, Prusa street 14, 08-110 Siedlece, Poland

The aim of our study was a detailed investigation of species composition of entomopathogenic fungi inhabiting forest litter and soil in pine forests in certain areas with different levels of air contamination with $S0_2$ and NO_x.

Samples of soil and forest litter from pine forests in 21 areas in Poland with different levels of air contamination with SO_2 and NO_x were collected in October 1993 and were studied for the presence of entomopathogenic fungi. The fungi were isolated from the samples at 18°C and 26°C with the test insects method using larvae of *Galleria mellonella* L. and *Achroia grisella* L. under laboratory conditions.

Larvae of the test insects introduced to the soil or to forest litter were found to be infected by five entomopathogenic fungi: *Beauveria bassiana, Metarhizium anisopliae, Paecilomyces* farinosus, *P. fumosoroseus* and *Verticillium* spp. Air contamination with SO_2 and NO_x does not eliminate entomopathogenic fungi from forest litter and soil however it affects the frequency of their isolation. *P. farinosus* proved to be the species most sensitive to air contamination while *P. fumosoroseus* and *B. bassiana* were the least sensible ones.

INTRODUCTION

In forest biotopes, forest litter and the upper layer of soil are the principal environment for entomopathogenic fungi that propagate on insects living therein permanently or those using the environment for pupation and overwintering (Kozłowska, 1956, Świeżyñska, Górnaś, 1976, Bałazy, 1981, Miętkiewski et al., 1991).

At the beginning of the vegetation season, the forest litter and soil are the principal source of infectious material from which mycoses spread in the environment. Fungal diseases in forest environment occur everywhere and are very important in the regulation of forest pest populations. They also create perspectives for the use of some species for biological pest control.

The aim of our study was detailed investigation of species composition of entomopathogenic fungi inhabiting forest litter and soil in pine forests in some areas with different levels of air contamination with SO_2 and NO_x.

MATERIAL AND METHODS

Samples of soil and forest litter from pine forests in 21 areas with different levels of air contamination with SO_2 and NO_x were collected in October 1993 and were studied for the presence of entomopathogenic fungi (fig.1). The fungi were isolated from the samples with the test insects method (Zimmermann, 1986) using larvae of *Galleria mellonella* L. and *Achroia grisella* L. 10 larvae were placed to each of 100 ml plastic containers filled with tested soil and forest litter. To reveal fungi species of different temperature requirements containers were incubated at 18°C and 26°C. Three containers were used in each variant of the analysis. Larvae mortality was assessed after 25 days of the incubation. The fungi were identified with the help of microscopic preparations made directly of mycelium developing on dead larvae in wet chambers. Where this was insufficient the fungi were transferred to standard media and cultivated until they developed features permitting their identification as to species or genus.

RESULTS

Larvae of the test insects introduced to the soil or forest litter were found to be infected by entomopathogenic fungi: *Beauveria bassiana* (Bals) Vuiill., *Metarhizium anisopliae* (Metsch.) Sor., *Paecilomyces farinosus* (Holm ex Gray) Brown et Smith, *Paecilomyces fumosoroseus* (Wize) Brown et Smith, and *Verticillium* spp. (tab. 1).

The fungus *B. bassiana* was most often found on the larvae of the test insects. It was more characteristic for forest litter than for soil (tab. 2). This species infected two times more larvae at 18°C than at 26°C (tab. 1) both in forest litter and soil. It was most often isolated in I/II and III classes of air contamination (tab. 2).

P. farinosus, the second species with respect to frequency of occurrence also more often infected insects at 18°C than at 26°C and was more characteristic for forest litter than for soil. This species more often infected insects in the samples from IV/V classes of air contamination.

M. anisopliae and *P. fumosoroseus* were found in the studied samples with similar frequency though less often than *B. bassiana* and *P. farinosus.* Both these species were more characteristic for soil than for forest litter and infected more larvae at 26°C than at 18°C. These species often infected a very high percentage of larvae in a sample, for example *M. anisopliae* in the soil from near Browsk (tab. 1). *P. fumosoroseus* was most often found in the samples from I/II classes of air contamination and sometimes from IV/V classes. *M. anisopliae* was most rare in III class of contamination.

A fungus of genus *Verticillium* was found in the studied samples. It was more often found on the larvae of test insects at 26°C than at 18°C. It was isolated the most from the samples from III class of contamination and the least from the samples from I/II classes of contamination.

Summarizing it can be said that air contamination with SO_2 and NO_x does not eliminate entomopathogenic fungi from forest litter and soil however it affects the frequency of their isolation. *P. farinosus* proved to be the species most sensitive to air contamination while *P. fumosoroseus* and *B. bassiana* were the least sensible ones.

I/II classes - 26 - 40 mg or above/m^3/year SO_2
31 - 40 mg or above/m^3/year NO_x

III class - 16 - 25 mg/m^3/year SO_2
26 - 30 mg/m^3/year NO_x

IV/V classes - 0 - 15 mg/m^3/year SO_2
0 - 25 mg/m^3/year NO_x

Fig. 1. Distribution of study sites in ralation to air pollution.

REFERENCES

BAŁAZY, S., 1981. Ściółka leśna ostoją grzybów owadobójczych. Las-Polski, 3: 14-15.

KOZŁOWSKA, Cz., 1956. Grzyby owadobójcze występujące na materiale pochodzącym z poszukiwań szkodników leśnych. Roczn. Nauk Leśn. 19: 43-61.

MIĘTKIEWSKI, R., ŻUREK, M., TKACZUK, C., BAŁAZY, S., 1991. Occurance of entomopathogenic fungi in arable soil, forest soil and litter. Roczn. Nauk Roln., s. E, 21: 61-68.

ZIMMERMANN, G., 1986. "*Galleria* bait method" for detection of entomopathogenic fungi in soil. Zeitch. angew. Entomol, 2: 213-215.

This study was done in the project of Forest Research Institute in Warsaw.

A LABORATORY STUDY ON INFECTION OF CEREAL APHIDS (*RHOPALOSIPHUM PADI* AND *METOPOLOPHIUM DIRHODUM*) WITH THE TWO HYPHOMYCETE FUNGI *BEAUVERIA BASSIANA* AND *VERTICILLIUM LECANII*.

Karsten Dromph, Susanne Vestergaard and Jørgen Eilenberg

Department of Ecology and Molecular Biology, Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark.

Summary

Two species of cereal aphids, *Metopolophium dirhodum* (Walker) and *Rhopalosiphum padi* (L.) were treated with isolates of *Beauveria bassiana* (Balsamo) Vuillemin or *Verticillium lecanii* (Zimmerman) Viegas. Both apterous and alates of *R. padi* were treated while only apterous *M. dirhodum* were treated. LC_{50}-values at day 9 were calculated and compared. The *V. lecanii* isolate had only significant effect on mortality of *M. dirhodum*, while *B. bassiana* had a significant higher LC_{50}-value on apterous *R. padi*, than on apterous *M. dirhodum*.

Introduction

In Danish winter wheat fields the following tree species of cereal aphids are most common: the birdcherry-oat aphid *Rhopalosiphum padi* (L.), the rose-grass aphid *Metopolophium dirhodum* (Walker) and the English grain aphid *Sitobion avenae* (Fabricius). The relative abundance's of the three species in the fields are, however, varying between years and between fields (Hansen 1995).

The aim of this study was to initiate laboratory infections among cereal aphids using *Beauveria bassiana* (Balsamo) Vuillemin and *Verticillium lecanii* (Zimmerman) Viegas and to obtain information about dose-response relationships.

Material and methods

Aphids. Stock cultures of the aphids were maintained at 20°C and 24 hour light on one-week-old winter wheat seedlings. Cohorts of newly moulted adults were used for the bioassays. Fifty adult apterous *M. dirhodum* or alate *R. padi* from the stock were introduced per pot. Each pot contained one-week-old winter wheat plants grown in vermiculite covered with water agar. The adult aphids were left on the plants for two days and then removed. Apterous *M. dirhodum* and alate *R. padi* were used when 9-10 days old, and apterous *R. padi* when 7-8-days-old.

Fungal isolates. Two isolates were used: one *V. lecanii* ,VL4, obtained from an unidentified Danish aphid and one *B. bassiana* obtained from *Limothrips cerealium* Haliday collected in a Danish winter wheat field and reisolated after transmission to a apterous *M. dirhodum*. Stock plates were stored at 10°C.

Conidial Suspension. The fungi were grown on SDA-plates for 14 days at 25°C. The spores were harvested using 0.05% Triton X-100. The suspension was first cleared for hyphal debris by

filtration and centrifugation. The conidia were then resuspended in 0.5 ml of 0.05% Triton X-100. The concentration of the suspension was determined using a hemacytometer and diluted to 10^4, 10^5, 10^6, 10^7 and 10^8 conidia per ml. The spore viability was examined by placing three droplets of the 10^7 conidia/ml. suspension on SDA droplets on microscope slides followed by incubation for 24 hours at 17.5°C. Germination was examined under light microscope, by observing 100 conidia on each SDA drop.

Bioassay. The aphids were inoculated by placing them singly in small plastic cups with 5 ml of water agar in the bottom, and with a 7-8 days old wheat seedling dipped in one of the spore suspensions or in 0.05% Triton X-100 placed in the agar. The cups were closed with 12 µm thick PVC cling film. 24 aphids were treated per each concentration, and the experiment was replicated three times.

The bioassays were carried out at 17.5°C/L17:D7 to mimic the average weather in July in the eastern part of Zealand, Denmark (Anon. 1975). The mortality was recorded daily for 14 days. After 7 days a fresh leaf was placed in each cup next to the old. Dead aphids were transferred to a new cup with water agar to allow fungal sporulation.

Data Analysis. Aphids that died at day one were excluded of the analysis, because the death probably was due to the handling. The mortalities at day 9 were analysed using the Probit analysis program by Michel Raymond, Laboratoire de Génétique, Institut des Science de L'Evolution U.S.T.L. France, and the LC_{50}-values were compared.

Results

The viability of the conidia was very high, >95%, in all the experiments justifying no need for adjustment of spore concentrations. The cumulative mortality are shown in figs 1-6. At day nine the control mortalities were still low, why the mortalities of this day were analysed. The LC_{50}-values for those aphid - pathogen combinations for which a regression line could be fitted are listed in table 1. There was no significant difference between apterous *M. dirhodum* treated with *B. bassiana* and with *V. lecanii*. The *V. lecanii* isolate had no significant effect on either of the morph's of *R. padi* (see figure 5 and 6). *B. bassiana* had some effect but was significant lower for the apterous *R. padi* than for apterous *M. dirhodum*. There was no significant difference between the LC_{50}-values of apterous and alate *R. padi*, but a weak tendency that the alates were more susceptible to infection than the apterous was observed.

Table 1. LC_{50} of *B. bassiana* and *V. lecanii* against *M. dirhodum* and *R. padi* at day 9.

Aphid	Pathogen	LC_{50}	95% FL [1]	
		conidia/ml	Lower	Upper
Apterous *M. dirhodum*	*V. lecanii*	$8.07*10^{4\ a}$	$1.11*10^4$	$2.78*10^5$
Apterous *M. dirhodum*	*B. bassiana*	$5.44*10^{5\ a}$	$1.54*10^5$	$1.80*10^6$
Apterous *R. padi*	*B. bassiana*	$8.07*10^{7\ b}$	$3.05*10^6$	$2.95*10^9$
Alate *R. padi*	*B. bassiana*	$3.02*10^{6\ ab}$	$1.20*10^6$	$7.03*10^6$

[1] 95% Fiducial limits.

LC_{50}-values followed by the same letter are not significantly different.

Discussion

The tested isolates of *B. bassiana* and *V. lecanii* have a low virulence against cereal aphids, since only at day 9 LC_{50}-values were of significance. Further, *M. dirhodum* was more susceptible to the tested fungi than *R. padi*. A low susceptibility to infection by these fungi has also been demonstrated by Feng et al. (1990). Such observations are in agreement with the lack of *B. bassiana* and *V. lecanii* to establish natural epizootics. Natural fungal control of cereal aphids is mainly due to entomophthoralen fungi like *Erynia neoaphidis* Remaud. et Henn. and *Entomophthora planchoniana* Cornu (Latteur, 1973; Feng et al. 1991; Steenberg & Eilenberg 1995). This is not only a result of the low susceptibility to infection with *B. bassiana* and *V. lecanii* but also due to the long lethal time, during which the aphids in this study continued to reproduce at approximately the same rate. This means that the fungal infection only will have a limited effect on the total number of offspring's per aphid, and thereby on the growth of the population.

There was no significant difference in the LC_{50}-values of apterous and alate *R. padi* treated with *B. bassiana*, but a tendency for the alates to be more susceptible to infection than the apterous. It has previous been shown that alates of the Pea Aphid, *Acyrthosiphon pisum* (Harris), are more susceptible to *E. neoaphidis* infections than apterous (Lizen et al. 1985). It is therefore possible that a difference in the susceptibility of the morph's of *R. padi* to *B. bassiana* infections is also present. However a more virulent isolate than the one used in this study is necessary to prove it.

Acknowledgement

This study was carried out as part of Centre for Agricultural Biodiversity supported by The National Environmental Research Programme.

References

ANONYMOUS, 1975. Middelnedbør og middeltemperatur. 1930-1960. Måned, år og vækstperiode. Det Danske Meteorologiske Institut. København. 1975.

HANSEN, L. M., 1995. Aphids - the nationale pest in Denmark. SP raport nr. **4**: 115-128.

FENG, M. G., JOHNSON J. B. & KISH L. P., 1990. Virulence of *Verticillium lecanii* and an Aphid-Derived Isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for Six Species of Cereal-Infesting Aphids (Homoptera: Aphididae). Environ. Entomol. **19**: 815-820.

FENG, M. G., JOHNSON J. B. & HALBERT S. E., 1991. Natural Control of Cereal Aphids (Homoptera: Aphididae) by Entomopathogenic Fungi (Zygomycetes: Entomophthorales) and Parasitoids (Hymenoptera: Braconidae and Encyrtidae) on Irrigated Spring Wheat in Southwestern Idaho. Environ. Entomol. **20**: 1699-1710

LATTEUR, G., 1973. Étude de la dynamique des populations des pucerons des céréales. Première données relatives aux organismes aphidiphages en trois localités différentes. Parasitica **29**: 134-151.

LIZEN, E., LATTEUR, G. & OGER. R., 1985. Sensibilité à l'infection par *l'Entomophthorale Erynia neoaphidis* Remaud. et Henn. du puceron *Acyrthosiphon pisum* (Harris) selon sa forme, son stade et son âge. Parasitica **41**: 163-170.

STEENBERG, T. & EILENBERG, J., 1995. Natural Occurrence of Entomopathogenic Fungi on Aphids on an Agricultural Field Site. Czech Mycology (In press).

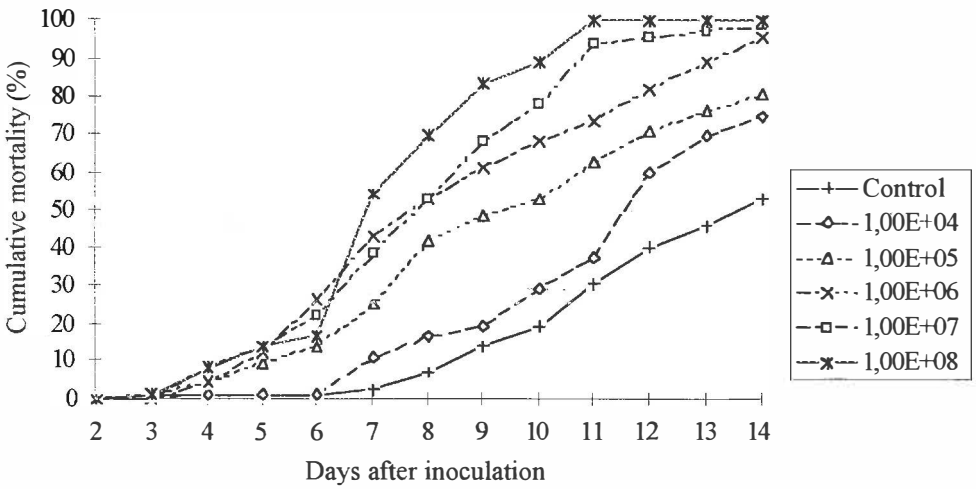


Fig. 1. Mortality of apterous *M. dirhodum* treated with *B. bassiana*.

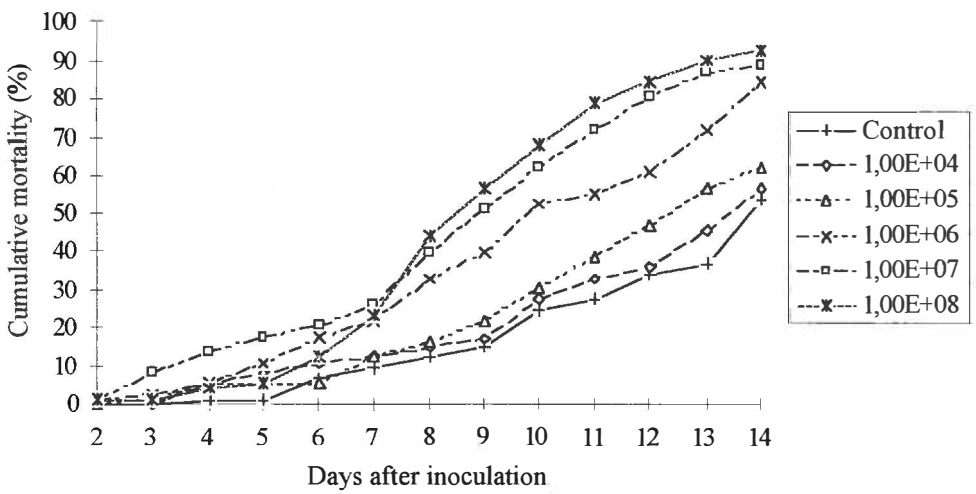


Fig. 2. Mortality of apterous *R. padi* treated with *B. bassiana*.

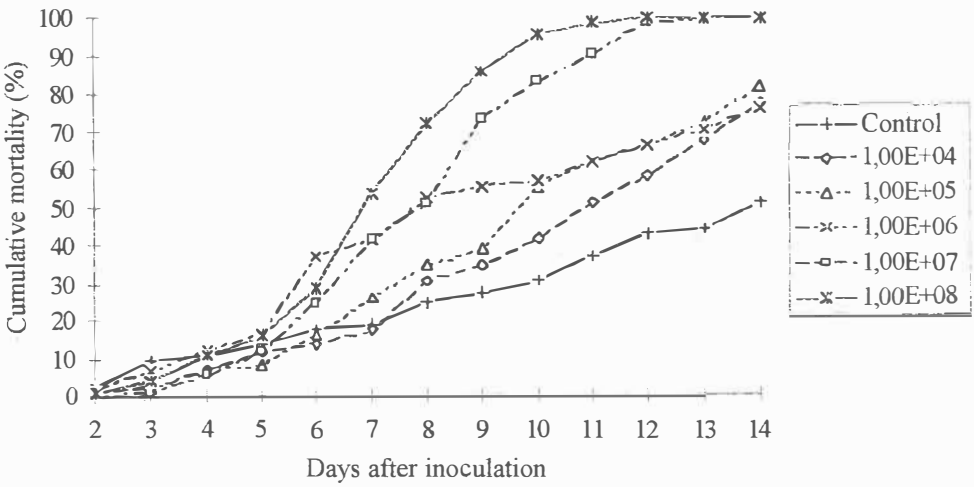


Fig. 3. Mortality of alate *R. padi* treated with *B. bassiana*.

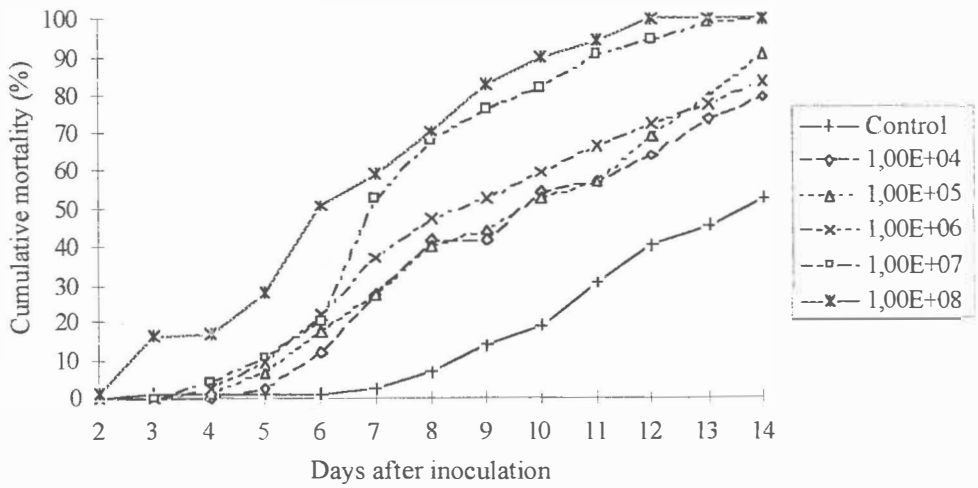


Fig. 4. Mortality of apterous *M. dirhodum* treated with *V. lecanii*.

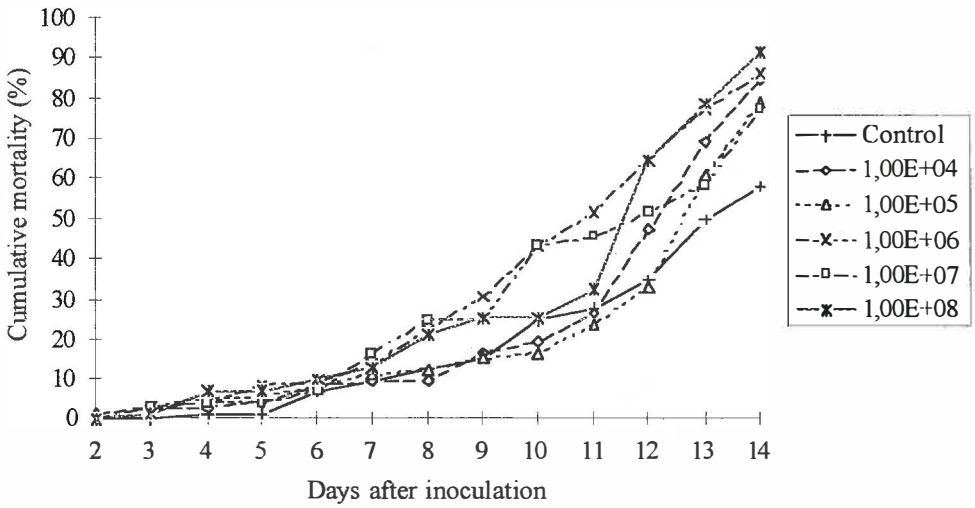


Fig. 5. Mortality of apterous *R. padi* treated with *V. lecanii*.

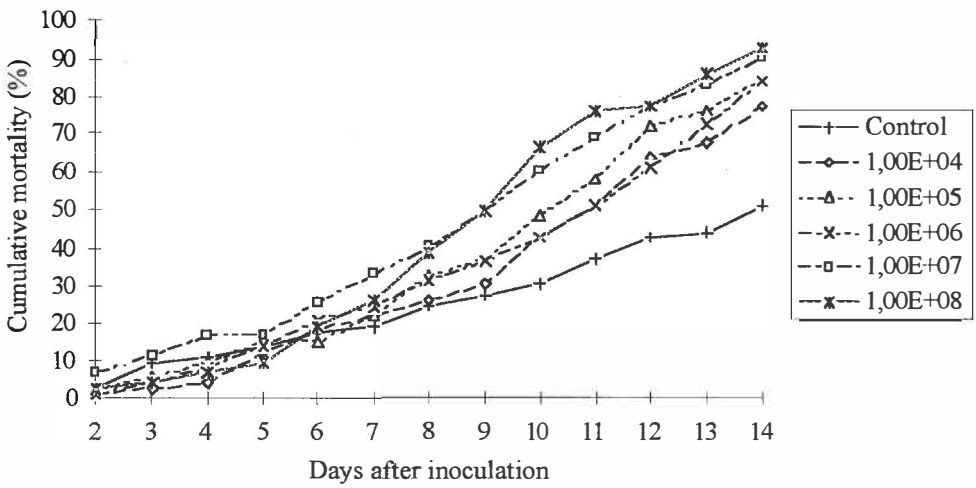


Fig. 6. Mortality of alate *R. padi* treated with *V. lecanii*.

EFFECT OF SOME FORMULATION SUBSTANCES ON GERMINATION AND VIRULENCE OF *BEAUVERIA BASSIANA* AND *VERTICILLIUM LECANII* CONIDIA

G.GALANI & A.-M. ANDREI

Research Institute for Plant Protection, Ion Ionescu de la Brad 8, 71592 Bucharest, Romania

Acceptance of entomopatogenic fungi as biological insecticides is decisively influenced by development of some dosable formulations applicable with conventional equipment (Bakker,1990; Laird, 1990). To this end, the fungal biomass will be formulated with various additives chemical compounds with protective, adhesive, antifoaming, wetting roles able to provide well defined formulations, stable, economically standardized and biological active (Couch et al., 1981). Assesment of compatibiliy between the pathogen and these compounds is a compulsory step, preceding their incorporation in formulation (Galani, 1986).

Seeking for some fungal formulations as water emulsifiable concentrate, we have studiate the effect of some pesticide emulsifiers on *Beauveria bassiana* and *Verticillium lecanii* cultures. For the tests it was selected products based on anionic, nonionic poliethoxylates and butanol surfactans, which showed no phytotoxic efect and are biodegradable, thus notpresenting danger of water pollution. To assess the toxic effect of compounds it was used two variants ; (1) spore suspension, i.e. vegetative mycelium + emulsifiers, have been seeded on a solid medium just after mixing, as well as after 24, 48 and 72 hrs; (2) the emulsifiers diluted in distiled water were impregnated in paper dishes which were placed onto agar medium seeded with acqueous conidia, and vegetative mycelium (Galani, 1980). Their influence on insecticidal action of fungal cultures was determinated by bioassay with *Leptinotarsa decemlineata* larvae (Ferron, 1981; Hall, 1981).

The analised emulsifiers showed, in general, a antimicrobian activity against both vegetative mycelium and conidia (table 1). As exceptions, UR and C-37 emulsifiers were inactive until 5%, first for both fungi and second for *V.lecanii* . P-55, R-55 and F-22 were also inactive for both fungi, but only at 0,5 % concentration. Between conidia and vegetative mycelium sensibility were not a great difference. In general, spors were more sensitive then vegetative mycelium.

table 1

E-mulsifier	Concentration % 5	3	1	0.5	0.2	0.1	5	3	1	0.5	0.2	0.1
	vegetativ mycelium						spors					
	B.bassiana											
C-37	+	-	-	-	-	-	+	+	+	+	+	-
F-22	+	+	+	-	-	-	+	+	+	+	-	-
F-46	+	+	+	+	+	+	+	+	+	+	+	+
NF-4	+	+	+	+	+	-	+	+	+	+	+	-
NF-8	+	+	+	+	+	+	+	+	+	+	+	+
P	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
P-55	++	++	+	-	-	-	++	++	+	-	-	-
R-55	+	+	-	-	-	-	+	+	+	+	-	-
UR	-	-	-	-	-	-	-	-	-	-	-	-
	V.lecanii											
C-37	-	-	-	-	-	-	-	-	-	-	-	-
F-22	++	++	++	-	-	-	++	++	++	-	-	-
F-46	+	+	-	-	-	-	+	+	-	-	-	-
NF-4	+	+	+	+	+	-	+	+	+	+	+	-
NF-8	+++	+++	+++	+	+	+	+++	+++	+++	++	++	++
P	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
P-55	+	+	+	-	-	-	+	+	-	-	-	-
R-55	+	+	+	-	-	-	+	+	-	-	-	-
UR	-	-	-	-	-	-	-	-	-	-	-	-
CAPTAN - 0.4 % : vegetative mycelium = +++ ; spors = +++												
CONTROL : vegetative mycelium = - ; spors = -												

(+ ... +++) = different level of fungic effect ; (-) = nonfungic effect

table 2

Emulsifier + er=0.1%	% Mortality after.....days *B.bassiana* 3	5	9	*V.lecanii* 3	5	9
C-37	2	81	87	5	34	80
F-46	11	83	90	3	51	82
UR	9	74	89	0	63	89
Tween-80	10	68	93	0	29	87
Fungal culture	13	63	94	2	58	81
Control	0	0	3	0	0	0

The bioassay of some emulsifiers + fungal cultures on the *L.decemlineata* (L_2 . L_3) larvae showed that virulence of both fungi were not affected, the biological activity was as high as control (table 2). As physical characteristics of formulation, it was noted that, due to their hydrophylous-lipophylous or polar character, molecules of emulsifiers achived a good dispersion in water of fungal spores, known as being stongly hydrophobes. Likewise, it is to note the fact that emulsions obtined, showing signaficance for the applicative technique, do not present strong foaming tendency, therefore they do not rise special problems in pulverisation. Also, contributing to lessening superficial tension of emulsions, the trialled emulsifiers provided fine and uniform pulverisation.

1. BAKER,R.R., AND DUNN,P.E. 1990. "New Directions in Biological Control". Alan R. Liss, New York.
2. COUCH, T. L., AND IGNOFFO, C. M. 1981. Formulation of insect patogen. *In* "Microbial Control of Pests and Plant Diseases 1970-1980" (H.D. Burges, ed.), pp. 621-634.
3. FERRON, P. 1981. Pest control by the fungi *Beauveria* and *Metarhizium*. *In* Mcrobial Control of Pests and Plant Diseases 1970-1980." (H.D. Burges, ed.), pp. 465-482. Academic Press, New York.
4. GALANI, G. 1980. Influenta unor pesticide asupra germinarii si cresterii ciupercilor entomopatogene *Verticillium lecanii*, *Paecilomyces farinosus* si *Beauvera bassiana*. An.ICPP, 16, pp.243-252.
5. GALANI, G. 1986. Influenta unor substante de conditionare asupra germinarii si patogenitatii ciupercilor *Verticillium lecanii* si *Paecilomyces farinosus* cultivate submers. An.ICPP, 19, pp. 99-117.
6. HALL, R.A. 1981. The fungus *verticillium lecanii* as a microbial insecticide against aphids and scales. *In* "Microbial Control of Pests and Plant Diseases 1970-1980." (H.D.Burges, ed.), pp. 483-498. Academic Press, New York.
7. LAIRD, M., LACEY, L.A., AND DAVIDSON, E.W. 1990. "Safety of Microbial Insecticides". CRC Press, Boca Raton, Florida.

EFFECT OF METAL IONS ON *PAECILOMYCES FUMOSO-ROSEUS*

M. Jaworska[1], A. Radkowska[1], D. Ropek[1] and P. Tomasik[2]

[1] Department of Agricutural Environment Protection, Academy of Agriculture, Mickiewicz Ave., 21, 31-120 Cracov,

[2] Department od Chemistry Academy of Agriculture, Mickiewicz Ave., 21, 31-120 Cracov, Poland

It is well known that entomopathogenic fungi are resistant to industrial pollution. On the other hand the entomopathogenicity of such fungi depends on biological and chemical factors. In our studies this common opinion is verified.

This presentation describes effect of the single metal ions on the growth and entomopathogenicity of *Paecilomyces fumoso-roseus.* The growth and entomopathogenicity of this fungus in respect to the culture grown on a pure solid agar was inhibited. Cr (VI), Ni (II), Pb (II) and V (V) lowered areas of colony 4-5 times. Tested metal ions effected on biological activity of *P. fumoso-roseus* too. Some of them, Cr (VI), Ni (II), Pb (II) and V (V), reduced entomopathogenicity of this fungus. An opposite effect exhibited Mg^{+2} ions.

Introduction

Presented results constitute an extension of our former studies on various organisms: *Escherichia coli* [Barabasz et al., 1990], *Saccharomyces cerevisia* [2], *Daphnia magna* [3], *Steinernema carpocapsae* [Jaworska et al., a, in press] and *Heterorhabditis bacteriophora* [Jaworska et al., b, c in press]. This presentation describes effect of the single metal ions on the entomopathogenicity of *Paecilomyces fumoso-roseus*.

It is well known that entomopathogenic fungi are resistant to industrial pollution. On the other hand the entomopathogenicity of such fungi depends on biological and chemical factors. In our studies this common opinion is verified.

Methods

Paecilomyces fumoso-roseus was inoculated on solid agar one of ion: AL (50 ppm), Cd (75), Co (II) (35), Cr (III) (35), Cr (VI) (35), Cu (II) (7.5), Fe (III) (200), Li (20), Mg (160), Mn (II) (400), Mo (VI) (100), Ni (II) (50), Pb (II) (200), Se (IV) (5.5), V (V) (105), Zn (200) added, and maintained at room temperature. Observation of an expansion of the fungus culture (in triplicate) were run for 8 days within 48 h intervals.

Then fungus samples collected after 4 weeks experiment were checked for their entomopathogenicity against adults of *Bruchus pisorum* L. in 2 h contact.

Results

The effect of metal ion on growth of *P. fumoso-roseus* in respect to the culture grown on a pure solid agar (control) was showed in Fig. 1. All tested metal ions inhibited growth (area of colony) of *P. fumoso-roseus.*

Metal ions lowered or even reduced (Cr VI, Ni, and V) entomopathogenicity of P. fumoso-roseus.

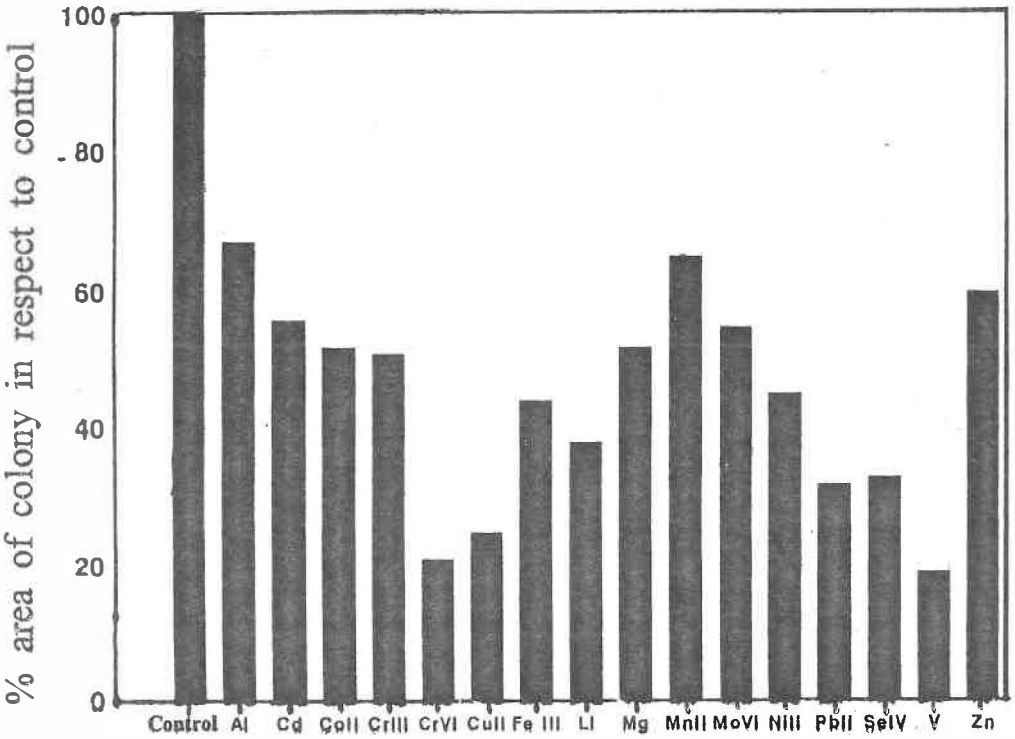


Fig. 1. The effect of metal ions on growth of *P. fumoso-roseus* in respect to the culture grown on a pure solid agar (control).

Conclusions

1. All metal ions under laboratory study inhibited the *P. fumoso-roseus* growth.
2. Tested metal ions reduced entomopathogenicity of *P. fumoso-roseus*.
3. Mg^{2+} ions exhibited an opposite effect. The mortality of *B. pisorum* L. was significantly higher in respect to the control.

References

Barabasz, W., Hetmańska, B., Tomasik, P., 1990,: Metal-metal interactions in biological systems, Part I, *Escherichia coli*, Water, Air, Soil Pollut., 52, 337-57.

Hetmańska, B., Tomasik, P., Tuszyński, T., 1994,: Metal-metal ınteractions in the biological systems, Part II, *Saccharomyces cerevisiae* ibid., 74, 281-288.

Tomasik, P., Magadza, C. H. M., Mhizha, S., Chirume, A., Metal-metal interactions in biological systems, Part III, *Daphnia magna,* ibid., in the press (after galley proof).

Jaworska, M., Sepioł, J., Tomasik, P., 1995a, Effect of metal ions on *Steinernema carpocapsae* entomopathogenic nematodes, ibid., in the press.

Jaworska, M., Gorczyca, A., Sepioł, J., Szeliga, E., Tomasik, P., 1995b, Effect of metal ions on *Heterorhabditis bacteriophora* entompathogenic nematodes, ibid., in the press.

Jaworska, M., Gorczyca, A., Sepioł, J., Tomasik, P., 1995c, Metal-metal interactions in biological systems. Part V, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora,* ibid., in press.

THE EFFECT OF AIR HUMIDITY ON THE DEVELOPMENT OF EPIZOOTICS CAUSED BY *ENTOMOPHTHORALES* IN POPULATIONS OF *MYZUS PERSICAE* (*HOMOPTERA, APHIDIDAE*)

I. MAJCHROWICZ, A. SEMCZYSZAK

Department of Entomology, Academy of Agriculture, Słowackiego 17,
71–434 Szczecin, Poland

The studies were conducted during the period of 4 years (1990–1994) in field conditions. The entomopathogenic fungi were investigated on *Myzus persicae*. The host plant was potato. An increase in air humidity at 3–10 p.m. brought sooner the appearance of epizooties in colonies of aphids (by 7–11 days) induced by *Entomophthorales*.
Myzus persicae were most frequently infected by *Erynia neoaphidis*. Other fungi species frequently infecting aphids in this experiment were *Entomophthora planchoniana* and *Conidiobolus obscurus*. Other pathogenic fungi of aphids sporadically found were: *Neozygites fresenii, Conidiobolus coronatus, Erynia phalloides* and *Erynia radicans*.
The results obtained suggest that prolongation of the retention of high humidity is one of the main factors influencing the course and the extent of epizootics caused by *Entomophthorales* in Myzus persicae population.

Introduction

As it is evident from literature (Missonier et al. 1970, Remaudière et al. 1981, Soper and MacLeod 1981, Keller 1991 and others) the occurrence of fungal infections of aphids is influenced by several kinds of factors, one of them being climatic (humidity, temperature, rainfalls , light) and second, high density of aphid population. The humidity, as it is known, particularly strongly favours the process of germination of the spores (Wilding 1969) and the latter's infecting of aphids. In literature (Bałazy et al. 1990, Elkassabany et al. 1992, Keller 1991, Kish et al. 1994 and others), most information deals generally with the effect of humidity on development of entomopathogenic fungi– causid epizooty in colonies of aphids. Our studies have been performed on 2 species of aphids: *Acyrthosophon pisum* (Majchrowicz and Semczyszak 1994) and *Myzus persicae*. These aphids differ among themselves significantly in biology and ecology. *A. pisum* appears in numerous compact colonies on the tops and lower parts of pea (and other legumes) leaves or on stem's top, while *M. persicae* are in smaller, dispersed, colonies mainly on the lower side of young and old leaves of many plants. The aim of the study was to investigate the influence of the length of a period with high air humidity on the course of *Entomophthorales* epizooty of *M. persicae*.

Material and methods

The studies were conducted during the summer season 1990, 1992–1994 in field conditions. The entomopathogenic fungi were investigated on *Myzus persicae*. The host plant was potato. The experiment was laid out in 5 replications in completely randomized arrangement. *M. persicae* obtained from *Capsicum annuum* cultivated in a glasshouse were transferred into each plot at a

density of 100 specimens, at the beginning of June. The study involved the influence of elevated humidity at 3–10 p.m. (created by installing foil insulators) exerted on the development of fungal epizooty in colonies of aphids, spectrum of *Entomophthorales* species attacking these insects. The control was made up of plots with natural variation of air humidity during 24 hours. The both variants of experiment were isolated from the natural invasion of *Aphidius sp.* as well as predators by means of insulators of nylon net of mesh dimensions 2x2 mm. A half randomly selected part of the plants investigated was covered with a foil isolator for a period from 3 p.m. to 8 a.m. That increased the humidity of the isolated plots almost to 100 %. During the other seven hours (8 a.m. to 3 p.m.), the air humidity within the plots of both combinations was the same.
M.persicae were counted on 20 leaves of potato. Both live and dead aphids were counted in each plot beginning from the first findings of aphids having been infected by *Entomophthorales.* At the early stage of the epizootic development, aphids were counted every third day. At the mass aphid mortality period, in the combination with an increased air humidity, aphids were counted every second day.
Additionally, at the same time 20 live aphids from each plot were collected and cultured on leaves of potato in Petri dishes for 4 days, as suggested in the literature . After this time the fungi were identified. Data obtained were processed by analyzing variance. Statistical differences were calculated using Tukey's test .

Results and discussion

In the combination with the prolongation of a high air humidity, the mycosis caused by *Entomophthorales* always occurred earlier than in the control plots. The statistical difference found between the number of aphids infected, coming from foil isolators and that from control plots persisted for 7 days in 1990 to 11 days in 1994 (Tab.1).
Myzus persicae were most frequently infected by *Erynia neoaphidis.* Other species of fungi frequently infecting aphids in this experiment were *Entomophthora planchoniana* and *Conidiobolus obscurus.* Next pathogenic fungi of aphids found sporadically were: *Neozygites fresenii, Conidiobolus coronatus, Erynia phalloides and Erynia radicans* (Tab.2).
The summer season in the year 1994 was exceptionally dry. Absence of rainfalls and high temperature in July exerted an inhibitory influence on the development of aphids and their fungal pathogens. It was only in the experiment with extended period of high air humidity in *M. persicae* colonies that there was a reduction of aphids caused by fungi. Positive influence, due to a long period of high air humidity on *E. neoaphidis* development was observed in dry or very dry years (1992, 1994) in remaining years (1990, 1993) that influence was less evident. As appears from Tab.1 a marked variation of the results was disclosed in particular years of the studies. However, in a similar experiment, concerning the influence of high humidity on *Entomophthorales* development in *Acyrthosiphon pisum* colonies, the variability of the results established in particular years of studies was insignificant (Majchrowicz and Semczyszak, 1994).
It is felt that the cause of the above–described differences is the varied structure of colonies being set up by *A. pisum* and *M. persicae* (which was pointed out in the method of studies) and biology of *E. neoaphidis* adapted to widespread dissemination and infecting aphids, particularly in high air humidity.
The second species of fungus that frequently appeared in colonies of *M. persicae* was *Entomophthora planchoniana.* It must be mentioned that this species was not detected on A. pisum at all in a similar experiment, which was accomplished in the years 1989–1992 Majchrowicz and Semczyszak, 1994). Some authors (Batko 1974, MacLeod et al. 1976, Bałazy 1994 and others)

classify *E. planchoniana* to the groups of fungi that are adapted to infecting aphids in medium conditions, and even in low air humidity. Remaudiere et al. (1981) reports that *E. planchoniana* offen causes death of aphids in tropical climate. That is also confirmed by our results of investigations obtained in 1993, being the coldest season in four years (Fig.1). At that time hardly some individuals of *M. persicae* were found to be infected by *E. planchoniana*. In the remaining years of studies this species differently reacted to the climatic conditions. Both the literature data (Missonier et al. 1970, Keller 1991, Remaudière et al.1981, Soper 1981, Kish et al.1994) and the results obtained in 1990 seem to indicate that *E. planchoniana* is recorded in larger amounts on aphids living in conditions of air humidity known to be lower during the daytime. However, when in 1994 the humidity was very low as a result of drought, that species was more frequently found on aphids from potatoes growing under conditions of prolonged period of high air humidity, and in control as well. The cause of variable reaction of this fungus in particular years to conditions existing during experiment should be looked for in its biological properties, structure and the shape of conidial spores, as well as some other adaptations to changeable environmental conditions. In the year 1994 markedly more individuals of *M. persicae* were confirmed to be infected by *E. planchoniana* than by *E. neoaphidis*, both in the experiment with prolonged air humidity and in the control.

The results obtained suggest that prolongation of the retention of high humidity is one of the main factors influencing the course and the extent of epizootics caused by *Entomophthorales* in *M. persicae* population.

Conclusions

1. Prolongation of a high air humidity period in the afternoon (3–10 PM) may accelerate by 7–11 days the occurrence, in *Myzus persicae* populations, of epizootics caused by fungi of *Entomophthorales*.
2. The most frequently occurring fungal pathogens of *Myzus persicae* are *Erynia neoaphidis* and *Entomophthora planchoniana*. They occur in significantly greater amounts on aphids living under conditions of prolonged periods of high humidity. This positive reaction is more distinct in *Erynia neoaphidis*.

References

1. Bałazy S., Miętkiewski R., Majchrowicz I. 1990. Mikozy mszyc – ich znaczenie i perspektywy wykorzystania w ochronie roślin. Zesz.Probl. Post. Nauk Roln. 392: 35–56.
2. Bałazy S. 1993. Fungi. Flora of Poland. Entomophthorales. Inst. Botaniki im. Szafera, PAN, Kraków, 24: 1–356.
3. Batko A. 1974. Filogeneza a struktury taksonomiczne Entomophthoraceae. Ewolucja Biologiczna. PAN, Ossolineum: 209–307.
4. Elkassabany N.M., Steinkraus D.C., MacLeod P.J., Correll J.C., Morelock T.E., 1992. Pandora neoaphidis (Entomophthorales: Entomophthoraceae): A potential biological control agent against Myzus persicae (Homoptera: Aphididae) on spinach. J. of the Kansas Entomol. Society 65(2): 169–1199.
5. Keller S. 1991. Arthropod – pathogenic Entomophthorales of Switzerland.II. Erynia, Eryniopsis, Neozygites, Zoophthora and Tarichium. Sydowia 43: 39–122.
6. Kish L.P., Majchrowicz I., Biever K.D., 1994. Prevalence of natural mortality of green peach (Homoptera: Aphididae) on potatoes and nonsolanaceous hosts in Washington and Idaho. Environmental Entomology 23(5): 1326–1330.

7. MacLeod D.M., Muller–Kogler E., Wilding N., 1976. Entomophthora species with E.muscae–like conidia. Mycologia Vol. LXVIII No 1: 1–29.
8. Majchrowicz I., Semczyszak A. 1994. The influence of air humidity on the development of epizootics caused by Entomophthorales in populations of Acyrthosiphon pisum Harris. Mat. z Konf. Nauk. PAN – Aphis and other Homopterous insects, Skierniewice 4: 101–107.
9. Missonier J., Robert Y., Thoizon G. 1970. Circostances epidemiologique semblant favoriser le development des mycoses a'Entomophthorales chez trois aphides, Aphis fabae Scop., Capitophorus horni Borner at Myzus persicae Sulz. Entomophaga 15: 163–190.
10. Remaudiere G., Latge J–P., Michel M–F. 1981. Ecologie comparee dees Entomophthoraceae pathogenes pathogenes de pucerons en France littorale et continentale. Entomophaga 26: 157–178.
11. Soper R. S., MacLeod D.M. 1981. Descriptive Epizootiology of an aphid mycosis. Tech. Bull. No 1632:: 1–17.
12. Wilding N. 1969. Effect of humidity on the sporulation of Entomophthora aphidis and E. thaxteriana. Trans. Br. Mycol. Soc. 53: 126–130.

This work was sponsored by the USDA (Grant: FG–Po–380).

Table 1.
The effect of humidity on development of mycoflora Myzus persicae (field experiments).

Date	No. of aphids (sum from 5 replications)				%M. persicae infected by fungi (mean from 5 replications)		LSD	Duration of significant differences
	Under isolator		without isolator					
	Total	infected by fungi	Total	infected by fungi	under isolator	without isolator		
(1990)								
10.07	7427	1842	8277	1448	25,20	16,68	9,74	
13.07	3246	874	7052	1741	31,00	26,46	20,55	
17.07	765	471	4745	1668	60,44	36,34	19,24*	7 days
19.07	306	165	3770	1268	63,60	33,36	27,69*	
24.07	113	39	1364	717	34,92	48,28	30,49	
(1992)								
31.07	767	83	1056	16	11,22	1,80	7,44*	
2.08	3021	535	3815	84	17,34	2,18	4,64*	
4.08	2621	1433	4736	433	56,34	9,62	16,26*	8 days
6.08	1134	942	4543	1114	84,16	24,60	13,59*	
8.08	222	62	326	107	24,62	31,22	34,28*	
(1993)								
16.07	8731	231	6093	29	2,76	0,46	2,53	
19.07	8935	318	7619	39	3,16	0,46	2,44*	
21.07	7500	852	7596	267	11,72	3,42	6,78*	9 days
23.07	6671	1524	8377	632	24,58	6,68	9,56*	
26.07	6632	2626	8737	1212	41,92	11,90	17,09*	
28.07	4511	1892	5130	1313	44,52	19,24	20,22*	
(1994)								
19.07	6257	152	5496	16	2,60	0,24	1,46*	
22.07	15025	894	15347	210	6,10	1,34	2,43*	
25.07	10131	1583	17646	343	16,64	2,00	7,37*	11 days
27.07	9040	3293	21784	225	36,58	1,00	10,82*	
29.07	4396	325	12989	95	7,50	0,64	3,33*	
1.08	4426	99	7016	14	3,52	0,40	3,54	

Explanation:
*– significant difference,

Table 2.
The effect of humidity on the development of mycoflora of Myzus persicae (experiment in Petri dishes).

Name of fungi	Year	Total	No. of aphids on 4th day culturing*** Dead individuals colonized by fungi under isolator	without isolator	Σ	F (ratio)
Erynia neoaphidis	1990	800	83	80	163	0,13
	1992	800	57	31	88	15,02*
	1993	800	93	71	164	0,76
	1994	800	77	1	78	45,30*
	Σ	3200	310	183	493	humidity 18,30* years 9,88* interaction 4,41*
Entomophtora planchoniana	1990	800	21	42	63	5,69*
	1992	800	14	15	29	0,05
	1993	800	1	6	7	–**
	1994	800	96	50	146	12,20*
	Σ	3200	132	113	245	humidity 0,24* years 33,11* interaction 16,11*
Conidiobolus obscurus	1990	800	6	15	21	–**
	1992	800	7	2	9	–**
	1993	800	86	44	130	2,61
	1994	800	18	1	19	–**
	Σ	3200	117	62	179	–**
●thers** fungi	1990	800	6	15	21	–
	1992	800	10	5	15	–
	1993	800	27	3	30	–
	1994	800	28	3	31	–
	Σ	3200	71	26	97	–

Explanation:
*– significant difference,
**– the differences are not significant becouse of low numbers of aphids infected with fungi,
***– sum from replications of periods of examination during a year.

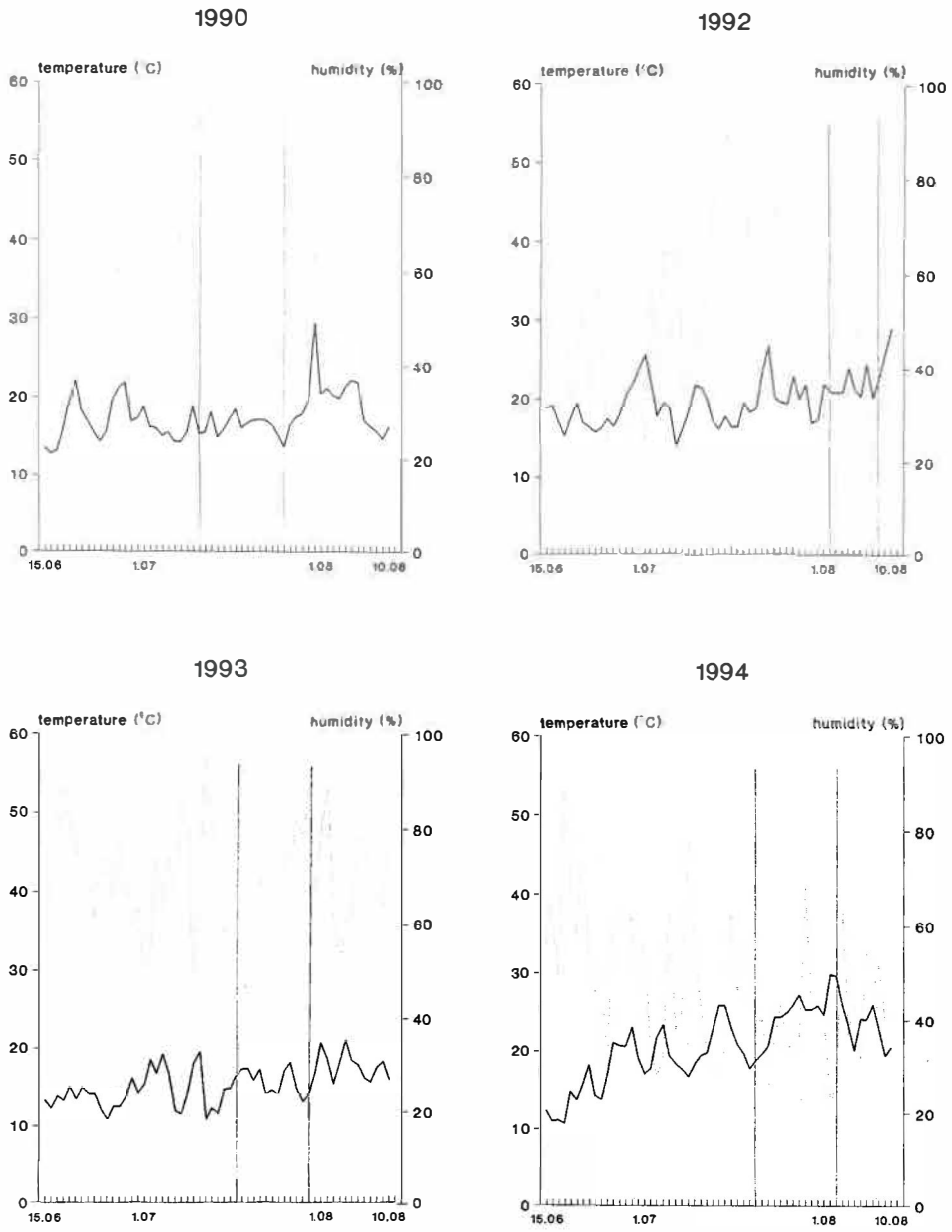


Fig. 1. Temperature and air humidity

LOCUST CONTROL WITH *METARHIZIUM FLAVOVIRIDE*: FORMULATION AND APPLICATION OF BLASTOSPORES

D. STEPHAN, M. WELLING AND G. ZIMMERMANN

Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, D- 64287 Darmstadt, Germany

Introduction

Metarhizium flavoviride is a well documented pathogen of locusts and a promising candidate for biocontrol of these pests. Recent studies on mass production, storability under tropical conditions and on the efficacy of formulations which allow a controlled droplet application at ultra low volume (ULV) rates evoked great interest (Lomer et al., 1993; Stathers et al., 1993). Mass production can be done in liquid culture by producing mycelium, mycelial pellets and hydrophilic blastospores or on the surface by producing aerial conidia (Zimmermann, 1993). For locust control we developed a submerged culture technique in order to produce high concentrations of blastospores (BS) using extremely cheap media. While storage of conidia is possible, only few investigations have been carried out on the storability of blastospores by drying (Fargues et al., 1979) or in liquid media (Kleespies & Zimmermann, 1994). Another aspect of our research was to develop a spray drying technique for blastospores of *M. flavoviride* and to study their viability during long-term storage.
Formulation and application of conidia in vegetable oils increase their efficacy against locusts compared to water-based formulations (Prior et al., 1988). In order to find a suitable ULV-formulation for spray-dried blastospores, a water-based formulation, an oil-based formulation and an oil-water emulsion was compared in semi-field trials in Mauritania.
The investigations were carried out within the framework of the GTZ-project "Integrated Biological Control of Grasshoppers and Locusts".

Material and methods

Fungus: The following isolates of *M. anisopliae* (Ma) and *M. flavoviride* (Mfl) were tested (isolate No., origin, host insect): Ma 97, Phillipines, unknown lepidoptera; Ma 106, Australia, *Austracris guttulosa* (Acrididae); Ma 131, Madagascar, soil sample; Mfl 5, Madagascar, *Locusta migratoria* (Acrididae); Mfl 6, Niger, *Ornitacris turbida cavroisi* (Acrididae). The isolates Ma 106 (= ARSEF 324) and Mfl 6 (= IMI 330189) were provided by Dr. R. Humber, USDA, Ithaca/USA and by Dr. C. Prior, IIBC, Ascot/UK, respectively.
Production of blastospores: In general, blastospores were produced in a standard liquid medium containing 2% glucose, 2% yeast extract, 1.5% corn steep and 0.2% Tween 80 modified according to Adamek (1963). For mass production, the following media were tested: 8% glucose and 8% of the following waste products: blood meal, horn meal, bone meal and fish meal, Naturpur™ (composted chicken droppings), Biosol™ (waste product of an antibiotic production) and a mixture of these six nitrogen sources.

Aliquots of 50 ml were inoculated with 5 x 10^7 BS. After three days incubation on a rotary shaker at 180 rpm and 25°C, the concentration of blastospores was determined using a Thoma cell chamber.
For mass production of blastospores (Mfl 5) in a laboratory fermenter (Infors), two liters of a waste product medium (4% of sugar-beet syrup + 8% Naturpur™) and the medium described by Adamek (1963) was used.
Spray-drying: Blastospores were suspended in deionized water and specific carriers. For spray-drying a Mini spray-dryer (Büchi) was used.
Determination of germination rate: The viability of spray-dried and freshly harvested blastospores was assessed by counting the germination rate after 8h (comparison of fresh and spray-dried BS) or 24h (storage of BS) at 25°C on a glucose/peptone/yeast extract agar containing antibiotics.
Storage: For long-term storage, spray-dried BS were incubated at different constant temperatures between 5°C and 50°C under dry, oxygen reduced conditions. The germination rates were assessed every two weeks as described above.
Semi-field trials: Semi-field trials were conducted at the field station of the GTZ in Akjoujt, Mauritania. Nymphs of *Schistocerca gregaria* (L_3/L_4) were treated with spray-dried BS corresponding to 4 x 10^{12} and 2 x 10^{13} BS per ha, respectively. For application, a MINI-ULVA (Manufacturer: MICRON) was used. Spray-dried BS were applied in three different formulations: A water based formulation (20% molasses and 80% water), an emulsion containing 40% TELMION™ (85% vegetable oil + 15% emulsifier; Manufacturer: Temmen, FRG) and 60% water, and an oil formulation (70% diesel fuel and 30% peanut oil). Treated larvae were transferred to field cages (2 x 2 x 2 m) in the natural habitat of the locusts. During the trials, the relative humidity ranged from 15 to 30% and the temperature from 10 to 35°C.

Results and discussion

PRODUCTION OF BLASTOSPORES

All waste products tested were suitable as nitrogen source for *M. flavoviride*. The highest numbers of BS were observed in media containing glucose and blood meal, Naturpur™, Biosol™ or the mixture (Table 1) with a blastospore concentration of up to 3.99 x 10^8. BS/ml. Corresponding to the results of Abu-Laban & Saleh (1992), waste products and especially animal manures are efficient nutrients for mass production of fungi. In a laboratory fermenter, the maximum yield of BS rose up to 1 x 10^9 BS/ml (medium: 4% molasses and 8% Naturpur™) and 2 x 10^8 BS/ml (medium described by Adamek, 1963) (Fig. 1). The advantage of the waste product medium based on molasses and Naturpur™ is that *M. flavoviride* produces mainly BS in high concentrations. The mycelial production is reduced. Therefore, a separation of blastospores from the mycelium is not necessary and the processing of blastospores is simplified.

SPRAY-DRYING

With the help of a laboratory spray-dryer a drying technique of blastospores was developed. The germination rate of spray-dried BS of different entomopathogenic fungi ranged from 88 to 90%. Only in Ma 131 the germination rate of spray-dried BS was 73%. The spray-drying of BS may lead to a slightly slower germination, but the pathogenicity is comparable to freshly produced BS (unpublished data).

STORAGE

After 52 weeks of storage at 5°C, 20°C and 30°C, the germination rate was 73.1%, 68.0% and 38.3%, respectively. Storage at 40°C and 50°C for about eight and two weeks, respectively, resulted in a loss of viability of 50%. For practical use the shelf-life of biopreparations has to be 12-18 months (Couch & Ignoffo, 1981). Our results have shown that storage at 5°C and 20°C is possible for at least one year. However, permanent storage temperatures of 30°C or more are not suitable for long-term storage. These findings correspond to the results of Stathers et al. (1993).

FORMULATION AND APPLICATION

BS have a hydrophilic cell wall and generally cannot be suspended in oil. Conidia of the genus *Metarhizium* have a lipophilic cell wall. Therefore, they can be suspended in oil-based ULV-formulations (Prior et al., 1988). However, after spray-drying, BS can be suspended in both water- and oil-based formulations. In semi-field trials, mortalities up to 100% were observed after application of the water based formulation containing 20% molasses and 80% water. Treatments with BS formulated in the oil-based formulation or the oil-water emulsion resulted in mortalities up to 70% (Fig. 2).

The results of the semi-field trials indicate that spray-dried BS formulated in a water-based formulation (20% molasses + 80% water) are able to kill desert locusts under arid conditions. Prior et al. (1988) assumed that water formulations do not adhere to the lipophilic cuticle of locusts. Therefore, spores might be lost before they could germinate and penetrate. However, molasses can also be used as an adhesive. One advantage of a molasses-based formulation is its safety to the environment. Furthermore, molasses is cheap and easy available. The results of this paper demonstrate that a biopreparation based on BS of *M. flavoviride* may be implemented in integrated locust control programmes in the future.

Table 1: Influence of different waste products as nitrogen sources (8%) and glucose (8%) on the blastospore concentration of Mfl 5

nitrogen source	BS x 10^8 ml $^{-1}$ (± s.e.m.)
mixture	3.99 (± 0.58)
Naturpur™	3.90 (± 0.74)
Biosol™	2.97 (± 0.14)
blood meal	2.88 (± 0.54)
bone meal	1.57 (± 0.45)
horn meal	1.29 (± 0.16)
fish meal	0.86 (± 0.26)

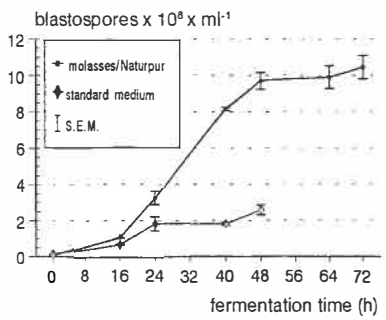
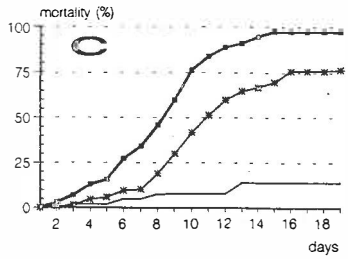
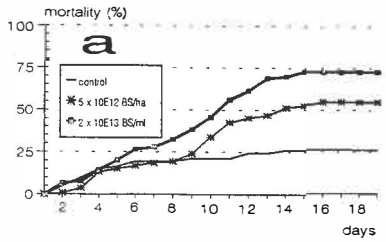
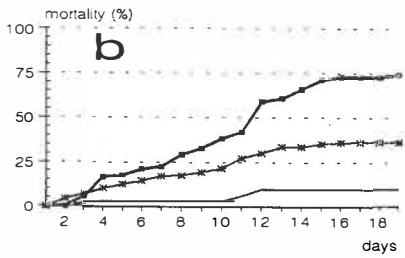


Fig. 1: Blastospore concentration of *M. flavoviride* during the fermentation process in the laboratory fermenter (Infors).
■: 4% molasses + 8% Naturpur™
◆: medium described by Adamek

Fig. 2: Comparison of different ULV-formulations of spray-dried BS of *M. flavoviride* Mfl 5 in semi-field trials in Mauritania:
(a) 40% Telmion^{TM} + 60% water
(b) 70% diesel fuel + 30% peanut oil
(c) 20% molasses + 80% water







References

Abu-Laban, A. Z., & Saleh, H. M., 1992. Evaluation of animal manures for mass production, storage and application of some nematode egg parasitic fungi. Nematologica **38**: 237-244.

Adamek, L., 1963. Submers cultivation of the fungus *Metarhizium anisopliae* (Metsch.). Folia Microbiologia (Praha) **10**: 255-257.

Couch, T. L. & Ignoffo, C. M., 1981. Formulation of insect pathogens. Pages 621-634 in Burges H D (ed) Microbial Control of pests and Plant diseases 1970-1980 New York: Academic Press

Fargues, J., Robert, P. H. & Reisinger, O., 1979. Formulation des productions de masse de l'hyphomycète entompathogène *Beauveria* en vue des applications phytosanitaires. Annales de Zoologie Ecologie Animale **11**: 247-257.

Prior, C., Jollands, P., le Patourrel, G., 1988. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidea). J. Invert. Pathol. **52**: 66-72

Kleespies, R. G., Zimmermann, G., 1994. Viability and virulence of BS of *Metarhizium anisopliae* (Metch.) Sorokin after storage in various liquids at different temperature. Biocontrol Science & Technology **4**: 309-319

Lomer, C. J., Bateman, R. P., Godonou, I., Kpindou, D., Shah, P. A., Paraïso A. & Prior, C., 1993. Field infection of *Zonocerus variegatus* following application of an oil-based formulation of *Metarhizium flavoviride* conidia. Biocontrol Science & Technology **3**: 337-346

Stathers, T. E., Moore, D. & Prior, C., 1993. The effect of different temperatures on the viability of *Metarhizium flavoviride* conidia stored in vegetable and mineral oils. J. Invert. Pathol. **62**: 111-115

Zimmermann, G., 1993. The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. Pestic. Sci. **37**, 275-379

FITNESS REDUCTION BY THE AFRICAN MIGRATORY LOCUST, LOCUSTA MIGRATORIA, AFTER APPLICATION OF LOW CONCENTRATIONS OF METARHIZIUM FLAVOVIRIDE BLASTOSPORES AND NEEM OIL

F. RANAIVO[1], M. WELLING[1], G. ZIMMERMANN[1] AND H. SCHMUTTERER[2]

(1) Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany
(2) Justus-Liebig-University Giessen, Institute for Phytopathology and Applied Zoology, Ludwigstr. 23a, D-35390 Giessen, Germany

Introduction

In previous works the entomopathogenic fungus *Metarhizium flavoviride* Gams & Rozsypal (Deuteromycetes; Moniliales) and products of the neem tree, *Azadirachta indica* A. Jussieu (Meliaceae), have shown to cause high mortality in *Locusta migratoria migratorioides* (Acrididae) (SCHMUTTERER et al., 1993; NICOL et al., 1994; WELLING et al., in press). However, little is known about their effects on the ecological fitness (activity), although a fitness-reduction may contribute to a successfull pest control under field conditions.
With respect to a combination of *M. flavoviride* and neem, the effect of both agents on *L. migratoria* was tested separately at low doses. The following parameters were recorded: (1) walking activity, (2) food consumption and (3) weight.

Material and methods

Products
Blastospores of *M. flavoviride*, strain Mfl5, were produced in a liquid medium and spray dried (STEPHAN et al., 1995). Germination rates checked before application were between 70 % and 90 % after 16 h on agar. Spore suspensions were prepared in a 50 : 50 petroleum : peanut oil formulation. Three concentrations (1×10^4, 5×10^4 and 1×10^5 spores ml^{-1}) were preliminary selected in a bioassay, corresponding to LC_{10}, LC_{40} and LC_{50} respectively.
Neem oil (Azadirachtin-content: 0.04 %) was obtained by cold-pressing of seed kernels and applied in a 50 : 50 petroleum : oil formulation. The oil phase was kept constant by adding peanut oil. Two concentrations were tested (5 %- and 12.5 %-neem oil), both corresponding to LC_{10} and LC_{50}.

Topical spray application
All experiments were carried out using gregarious 4th instar nymphs of *L. migratoria*. The different formulations were applied by means of a Grapho spray gun, using a nozzle of 0,3 mm diameter at a pressure of 2 bar. After spraying, the nymphs were transfered into cages in groups of 32 per treatment and incubated in a light-thermostat at 35° C (day)/ 25° C (night) and 12 : 12 h dark/light period.

Walking activity
The walking activity of the hoppers was examined every second day until the 10th day after treatment by means of a walking-arena.This was a 60 x 60 by 4 cm high wood cage, covered with a plexiglas-top with a circular gauze lid each in two corners. The white-painted roof was divided into numerated squares, thus allowing a rapid localisation of the

test insects during experiments. The walking-arena was placed in a room at 30 ± 1° C and illuminated with two 400-watt lamps. The relative humidity was 45 ± 10 %.
At the beginning of each experiment, 20 nymphs from the same treatment were placed in the walking-arena, 10 were marked. The path of each painted hopper was plotted on a scaled diagramm of the arena floor for 1 min out of every 30 min within 2½ h. The lenghts of the walking-tracks were measured with a map measurer, then used as quantitative measures of the walking activity.

Food consumption and weight
The food consumption was calculated by weighing the daily collected dry faeces. Furthermore, the nymphs were weighed every second day until the 10th day after the treatment.

Results and discussion

Walking activity
In the control hoppers, resting periods and walking periods were alternating in a definite pattern.The highest walking activity was recorded in the middle of each instar period (L_4 and L_5), while hoppers hardly moved on the day before and after moulting.

Table 1: Walking activity of nymphs of *L. migratoria* in % of the control after treatment with *M. flavoviride* or neem.

days after treatment	1 x 10^4 sp. ml^{-1}	5 x 10^4 sp. ml^{-1}	1 x 10^5 sp. ml^{-1}	5 %-neem	12.5 %-neem
2	116 %	120 %	133 %	74 % *	49 % *
4	58 % *	48 % *	47 % *	100 %	53 % *
6	94 %	75 % *	52 % *	53 % *	24 % *
8	89 %	53 % *	40 % *	21 % *	4 % *
10	92 %	44 % *	31 % *	24 % *	7 % *

* significant difference to the control by $\alpha = 0.05$

With increasing concentrations of blastospores the treated nymphs showed clear differences in their activity compared to the untreated nymphs. On the 2nd day after the treatment they became restless and hectic, in some cases also a sudden death was recorded. Within this restless period the walking activity was higher than that of the control but not significantly (Tab. 1). A restless phase is a typical symptom after fungal infection (BURNSIDE, 1930). It might act as a thermoregulating behaviour, with which the insect tries to reduce its increased body temperature due to an augmented energetic requirement for the immun system.
From the 4th day to the end of the experiment walking progressively decreased by the nymphs treated with blastospore concentrations corresponding to a LC_{40} and LC_{50} of *M. flavoviride*. They became more and more weary so that their walking activity compared to the control was 43 % and 31 %, respectively, after 10 days(Tab. 1). LEPESME (1938) observed a paralysis of the wings and legs in adults of *L. migratoria* infested by *Aspergillus flavus* and explained it with the mechanical decomposition of the muscles. In addition, MÜLLER-KÖGLER (1965) supposed a toxical effect. Among fungal metabolites, destruxins (DTX) are assumed to be the principal agent causing muscle atrophy and paralysis (CHARNLEY, 1990, 1992). SAMUELS (1988a, 1988b) described a depolarisation of the muscle membranes caused by DTX from *M. anisopliae* by different species of lepidoptera and diptera, resulting in paralysis and death.

After treatment with a blastospore concentration corresponding to the LC_{10} the walking activity was significantly lower on the 4th day and only slightly reduced on the following days compared to the control (Tab. 1). Possibly, too low concentrations may cause a temporary weakness of the nymphs followed by a convalescence.
Already on the 2nd day after neem application, the walking activity of the treated nymphs decreased significantly to 24 % (5 %-neem) and 7 % (12.5 %-neem) after 10 days compared to control (Tab. 1). Resulting fifth instar nymphs showed morphological defects on the legs, ranging from being bow-legged to loss of whole extremities, and on different parts of the body. These damages clearly enhanced the loss of mobility by the treated nymphs and may perhaps explain the stronger effect of neem compared to *M. flavoviride.*
The mechanism of the influence of neem on the mobility of locusts is not really understood. WILPS et al (1992, 1993) noted a reduced flight activity by *Schistocerca gregaria* after neem application. The authors suggested an influence on the hormonal activity of the corpora cardiaca, thus inhibiting the mobilisation of reserves from the fat bodies. Although locusts use carbohydrates as source of energy while walking, this model might be valuable to explain the loss of walking activity too.

Food consumption and weight
A significant reduction of the food intake was observed in all *M. flavoviride* treatments, depending on the blastospore concentration used. This inhibition was constant during the whole experiment after application of 1×10^5 spores ml^{-1}, while the food consumption increased after moulting in the two lower concentrations, probably due to a convalescence(Tab. 2). However, the total amount of faeces within 10 days (with increasing dose 81 %, 69 % and 28 % compared to the control) was significantly reduced in all treatments. A significant deficit of 118 %, 133 % and 197 % compared to the control was recorded after 10 days in the weight increase of the treated nymphs. Loss of feeding activity is a non-specific symptom of many fungal infections (MADELIN, 1963; MÜLLER-KÖGLER, 1965). MOORE et al (1992) also observed a reduction of food intake after application of *M. flavoviride* conidia.
Feeding inhibition is a well documented aspect of the influence of neem on locusts (SIEBER & REMBOLD, 1983; MORDUE (LUNTZ)et al., 1985; FREISEWINKEL, 1993). In these studies, feeding was strongly reduced after neem application, although the merely moulted individuals in the 5 %-treatment showed more appetite. Especially the nymphs in the 12.5 %-treatment nearly stopped their feeding activity (Tab. 2). Within 10 days, the total amount of faeces was 49 % (5% neem) and 16 % (12,5% neem) compared to the control, thus indicating a stronger feeding inhibition than with *M. flavoviride*. Ten days after application of neem, a total deficit of 116 % and 194 %, respectively, was observed in the body weight increase.

Table 2: Food consumption of nymphs of *L. migratoria* in % of the control after treatment with *M. flavoviride* or neem.

days after treatment	1×10^4 sp. ml^{-1}	5×10^4 sp. ml^{-1}	1×10^5 sp. ml^{-1}	5 %-neem	12.5 %-neem
2	116 %	100 %	83 %	78 % *	47 % *
4	61 % *	61 % *	30 % *	45 % *	14 % *
6	73 % *	63 % *	56 % *	53 % *	29 % *
8	62 % *	40 % *	17 % *	23 % *	10 % *
10	91 %	80 % *	11 % *	64 % *	5 % *

* significant difference to the control by $\alpha = 0.05$

Acknowledgements

These studies were carried out within the framework of the GTZ-Project "Integrated Biological Control of Grashoppers and Locusts". We would like to thank Dietrich STEPHAN for providing blastospores and Deborah GLOEGE for technical collaboration.

References

BURNSIDE, C. E. (1930). Fungous diseases of the honeybee. U.S. Dept. Agric. Techn. Bull. **No. 149**, 42 pp.

CHARNLEY, A. K. (1990). Secondary metabolites, toxins and entomopathogenic fungi: an evolutionary perspective. In: *Proceedings and abstracts, Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia, 20-24 August 1990.* Glen Osmond, Australia; Dept. Of Entomology, Univ. of Adelaide, 303-307.

CHARNLEY, A. K. (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: LOMER, C. J. & PRIOR, C. (eds), Biological Control of Locusts and Grasshoppers. *Proceedings of a Workshop held at the International Institute of Tropical Agriculture, Cotonou, Republic of Benin, 29 April-1 May 1991.* C.A.B. International in association with I. I. T. A, 181-190.

FREISEWINKEL; D. C. (1993). Orale und topikale Wirkungen des Samenöls des Niembaumes *Azadirachta indica* (A. Juss.) auf Larven der Afrikanischen Wanderheuschrecke *Locusta migratoria migratorioides* (R. & F.). Mitt. Dtsch. Ges. Allg. Angew. Ent. **8**, 785-789.

LEPESME, P. (1938). Recherches sur une Aspergillose des Acridiens. Bull. Soc. Hist. Naturelle Afr. Nord **29**, 372-384.

MORDUE (LUNTZ), A.J.; COTTEE, P. K. & EVANS, K.A. (1985). Azadirachtin: its effect on the gut motility, growth and moulting in *Locusta.* Physiol. Ent. **10**, 431-437.

MOORE, D.; REED, M.; LE PATOUREL, G.; ABRAHAM, Y. J. & PRIOR;C.(1992). Reduction of feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride.* J. Invertebr. Pathol. **60** (3), 304-307.

MÜLLER-KÖGLER, E. (1965). Pilzkrankheiten bei Insekten. Paul Parey, Berlin & Hamburg. 444 pp.

NICOL, C. M. Y.; ASSADSOLIMANI, D. C. & LANGEWALD, J. (1994). Caeliferae: short-horned grasshoppers and locusts. In: SCHMUTTERER, H. (Hrsg.), The Neem Tree *Azadirachta indica* (A. Juss.) and Other Meliaceous Plants. VCH Publishers, Weinheim.

SAMUELS, R. I.; CHARNLEY; A. K.; REYNOLDS, S. E. (1988a). Calcium channel activation of insects muscles by destruxins, insecticidal compounds produced by the entomopathogenic fungus *Metarhizium anisopliae. Comparative Biochemistry and Physiology* **90C**, 403-412.

SAMUELS, R. I.; CHARNLEY; A. K.; REYNOLDS, S. E. (1988b). The role of destruxins in the pathogenicity of 3 strains of *Metarhizium anisopliae* for the tobacco hornworm *Manduca sexta. Mycopathologia* **104** (1), 51-58.

SCHMUTTERER, H; BAUMGART, M.; FREISEWINKEL, D.; LANGEWALD, J. & NICOL, C. M. Y. (1993). The effects of neem oil and other neem products on nymphs and resulting adults of *Schistocerca gregaria*, *Nomadacris septemfasciata, Locusta migratoria migratorioides*, and *Zonocerus variegatus*. J. Appl. Ent. **116**, 178-176.

SIEBER, K.-P. & REMBOLD, H. (1983). The effects of azadirachtin on the endocrin moulting in *Locusta migratoria.* J. Insect Physiol. **29**, 523-527.

STEPHAN, D.; WELLING, M. & ZIMMERMANN, G.(1995). Locust control with *Metarhizium flavoviride*: formulation and application of blastospores. (See same IOBC Bulletin).

WELLING, M.; NACHTIGALL, G. & ZIMMERMANN, G. *Metarhizium spp.* isolates from Madagascar. Morphology, and effect of high temperatures on growth and infectivity to the migratory locust, *Locusta migratoria.* Entomophaga (in press).

Control of western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) in gerbera by incorporating the entomopathogenic fungus *Metarhizium anisopliae* into the growth medium

S. Vestergaard[1,2], A. T. Gillespie[1] and J. Eilenberg[2]
[1] Chr. Hansen's Bio Systems, 10-12 Bøge Alle, 2970 Hørsholm, Denmark. [2] Royal Veterinary and Agricultural University, Dept. of Ecology and Molecular Biology, Bülowsvej 13, 1870 Frb. C, Denmark.

Summary

The efficacy of the entomopathogenic fungus *M. anisopliae* against the western flower thrips *F. occidentalis* was assessed in the glasshouse by mixing fungal spores into peat containing gerbera plants.
Absolute and relative methods of monitoring thrips and fungus were used. Two out of three monitoring methods showed a reduction in the western flower thrips population 4 - 6 weeks after treatment with *M. anisopliae*, after which the thrips population increased rapidly. A decline in the number of *M. anisopliae* conidia per gram of soil was observed during the experimental period and this may have affected the control of western flower thrips.

Introduction

The western flower thrips *Frankliniella occidentalis* was introduced into Europe in the 1980's and is a pest of global economic importance in both field and protected crops (Bryan & Smith, 1956; Yudin *et al.*, 1986). Western flower thrips (WFT) damage crops directly through feeding and indirectly by transmitting viruses (Marchoux *et al.*, 1991). Control of WFT is often difficult because the insect has developed resistance to many insecticides and due to its life cycle, which includes two pupal stages normally occurring in the soil and the cryptic behaviour of adults and larvae which are generally found in flowers or in growing terminals. Much effort has been used to develop biological control agents against WFT on glasshouse crops. The predators *Orius* spp. and *Amblyseius* spp. can control thrips on vegetable crops but are often less successful on ornamental crops (Brødsgaard, 1991a; Tavella *et al.*, 1991; Ravensberg *et al.*, 1992).

Laboratory bioassays have indicated that several isolates of the entomopathogenic fungi *Metarhizium anisopliae* (Metsch.) are highly virulent to adult WFT (Vestergaard *et al.*, 1995) and semi-field experiments have revealed that the fungus is infective to WFT pupal stages in sphagnum (Vestergaard, 1995).

M. anisopliae has been isolated from soil and diseased insects throughout the world (Domsch *et al.*, 1980; Zimmerman, 1993) and the fungus has gained attention as a potential control agent for soil-inhabiting insects on outdoor crops, e.g. the pecan weevil *Curculio caryae* (Gottwald & Tedders, 1984), and pasture scarab *Adoryphorus couloni* (Rath, 1992). *M. anisopliae* has been used on a limited scale to control pest insects in the developing world for many years (Ferron, 1981) and products based on *M. anisopliae* are now in the process of registration in the USA and Australia (Miller, 1994; Bullard *et al.*, 1993).

The glasshouse environment is particularly suited to the use of *M. anisopliae* in the soil because of the relatively high temperature (without large fluctuations) and the high water content compared to field situations. WFT pupae infection is then normally not restricted by a lack of moisture as is the case with foliar applications of the fungus. However, only relatively

few experiments have been carried out regarding the role of *M. anisopliae* controlling soil-inhabiting insects in glasshouses, e.g. on the black vine weevil *Otiorhynchus sulcatus* (Zimmermann, 1984; Reinecke *et al.*, 1990; Moorhouse *et al.*, 1993).

The aim of this study was to determine the efficacy of *M. anisopliae* in controlling *F. occidentalis* in glasshouses when the fungus was incorporated into sphagnum. The persistence of the fungus in sphagnum was also assessed using a plate counting method.

Material and methods

Preparation of inoculum and plants

Conidia of the isolate *M. anisopliae* 275, originally isolated from *Cydia pomonella* by Zimmermann, Damstadt, Germany, were produced on autoclaved millet grains in polystyrene bags, 22.5 x 56 cm (Van Leer Packaging, Poole, UK) incubated at 26°C for 14 days. Conidia on millet grains were ground for 15 s in a coffee grinder before being incorporated by hand into a standard sphagnum (Unimuld, Sweden) at two doses: 1) 6×10^{11} conidia per m^3 sphagnum (=1000 l), and 2) 3×10^{12} conidia per m^3 sphagnum. Conidia viability was 99% as determined on Sabouraud dextrose agar (Oxoid, Basingstoke, U.K.) using the method of Hall (1976).

Plants, *Gerbera jamesonii* var. Festival, were produced from seed and when 6 weeks old they were repotted in 0.55 l pots and grown in sphagnum with or without *M. anisopliae*. Each group of 100 plants was arranged in three separate glasshouse chambers at The Danish Institute of Plant and Soil Science, Lyngby, DK on a table with plastic and capillary matting. The plants were watered automatically and fertilized according to the influx with ebb and flow system. In the last three weeks two tables were used for plants due to their enlarged size. During the experimental period from June 6 to August 24 1994 the average temperature was 22 to 24°C, the minimum temperature was ca 17°C and the maximum temperature was 37°C (due to an unusually warm summer). Relative humidity (r.h.) ranged between 40 and 95%. Aphids and whitefly were controlled by *Aphidius* spp. and *Encarsia formosa* respectively One week after repotting, *F. occidentalis* (3 imago, 2:1 ♀:♂) was released per plant.

Monitoring

Two weeks after the release of thrips, the insect populations in the three chambers were monitored once a week:

1. Adults were trapped using blue sticky traps (10 x 25 cm, Horiver TR, Koppert B.V., Berkel en Rodenrijs, Holland) which were hung 10 cm above the culture. One sticky trap per chamber was used in the beginning of the experiment and two were used from August 3 when the plants were moved on to two tables.

2. Thrips larvae were counted on two leaves (one young and one old) from 20 plants per treatment.

3. Larvae and adults in ten flowers were counted from the end of July.

4. The number of thrips pupating on capillary matting and plants was determined.

5. Ten plants from each treatment were qualitatively assessed for damage caused by *F. occidentalis*. A scale from 0 - 5 was used to assess the feeding marks on the petals where 0 was a measure for no damage, 1 was 1 - 20%, 2 was 21 - 40%, 3 was 41 - 60%, 4 was 61 - 80% and 5 was > 80% damage.

6. The prevalence of *M. anisopliae* in thrips populations was determined from approximately 25 adults trapped from five random leaves or flowers and placed on a chrysanthemum leaf in a chamber with high humidity and incubated at 23°C for five days. Dead insects were removed

daily and placed on a moistened filter paper in a Petri dish and examined for signs of *M. anisopliae* infection.

The persistence of *M. anisopliae* conidia in the sphagnum was examined over the entire experimental period. Sphagnum samples of one g were removed at depths of 2-3 cm from three random pots from each treatment. These samples were mixed with 9 ml 0.05% Triton X-100 (Sigma chemical Co., St. Louis, USA). 100 µl of a further diluted suspension was then pipetted onto three plates with a selective medium (Sabouraud dextrose agar with the addition of 0.5 ml l^{-1} Dodine/Radspor FL (Truchem Limited, Nottingham, U.K.), 50.0 mg l^{-1} Chloramphenicol, 50.0 mg l^{-1} Streptomycin sulphate). The plates were incubated at 23°C, colony forming units (cfu) were counted after five days and cfu g^{-1} dry weight sphagnum was determined.

Results

The number of larvae on *M. anisopliae* treated plants was significantly lower than on the untreated plants in week 6, and in weeks 4 and 5 the number was also lower but the different was not significant (Table 1). From week 6 - 9, the number of larvae and adults in flowers was higher in the untreated than in the *M. anisopliae*-treated plants, where it was reduced by up to ca 40% (Fig. 1). However, after nine weeks no significant difference was present between the untreated plants and those treated with a high dose of *M. anisopliae* (Fig.1). Sticky trap monitoring also showed that there were fewer thrips on treated plants in most weeks (Fig. 1). After week five, plants began to flower and the population of *F. occidentalis* increased dramatically, probably due to the available pollen in the flowers as well as higher temperature. The population of WFT increased relatively more in the treatment with the high dose of *M. anisopliae* after week six and the number of larvae was higher than the control (Table 1).

The qualitative damage on flowers caused by *F. occidentalis* was ca 20% on the control plants and 6 and 15% respectively for the lowest and highest doses of *M. anisopliae*. The flower damage of 6% was significantly lower than damage on the control plants.
In the last week (9) of the experiment, several thrips pupae were found on the underside of the leaves nearest the soil. In plants with the highest dose of *M. anisopliae*, 39 pupae were observed on ten plants and 19 thrips pupated on the plants with the lowest dose of *M. anisopliae*, while the lowest number of pupae was observed in the control. No thrips pupae were found under the pots or on the capillary matting.

The number of *M. anisopliae* cfu declined gradually in both treatments during the experimental period. The initial concentration of conidia was 2.4×10^7 conidia g^{-1} dry weight sphagnum for the low dose treatment. From week 6 the conidial number was reduced to approximately half (Table 2) and in week 9 the number was about ten times lower than the original number. The number remained high for a longer period in sphagnum treated with high doses of *M. anisopliae* conidia than for the lower dose treatment, but by week 6 the conidial number was also reduced by half. By week 9 the conidial number was approximately equal to the low dose treatment (Table 2).

The prevalence of *M. anisopliae* in the thrips population of the two treatments did not differ and in both treatments the prevalence varied between 4 and 12%. None of the thrips on the untreated plants were found to be infected with *M. anisopliae*.

Table 1. Mean number of *F. occidentalis* larvae on two leaves from 20 gerbera plants ± SE

Weeks	1	2	3	4	5	6	7	8	9
Control	4.1±1.0	0.7±0.2	4.4±0.9	5.8±0.9	3.5±0.7	6.8±1.5	9.0±2.1	5.9±1.3	8.5±2.4
Low dose	4.9±1.4	1.1±0.3	3.4±0.5	4.1±0.8	2.9±0.9	3.1±0.8	1.7±0.7	2.4±0.7	6.5±1.8
High dose	4.7±1.0	2.1±0.6	4.4±0.6	2.5±0.6	2.0±0.8	3.5±0.5	7.5±1.5	20.9±8.5	13.9±3.0

Control = untreated plants, low and high dose = *M. anisopliae* treated plants

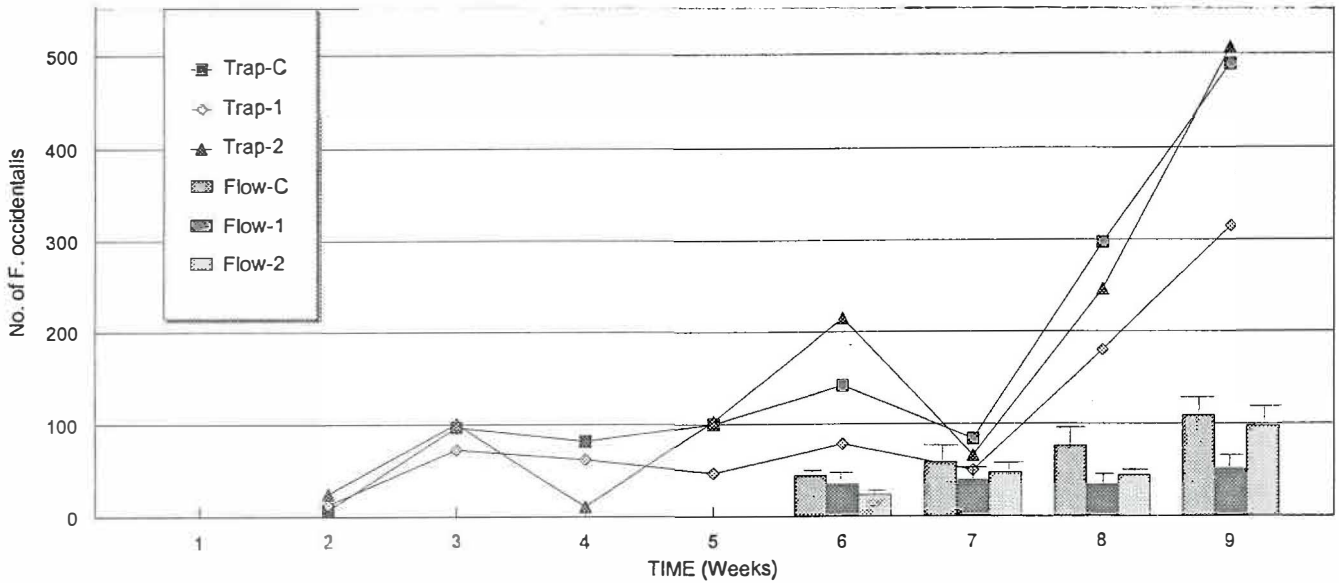


Figure 1. Population of *Frankliniella occidentalis* on gerbera plants after treatment with *M. anisopliae*.
Trap: the number of adult thrips caught on blue sticky traps (lines). **Flow**: mean number of adults and larvae counted on a flower ± SE (bars). C = untreated plants, 1 = low dose of *M. anisopliae* applied to the sphagnum, 2 = high dose of *M. anisopliae* applied to the sphagnum. Week 1 = June 28.

Table 2. Mean number of *Metarhizium anisopliae* conidia g^{-1} dry weight sphagnum of potted gerbera (three samples per treatment ± SE)

Weeks	0	1	2	3	4	5	6	7	8	9
low dose	24[a] ± 13	22 ±2.7	5.2 ± 0.7	16 ± 6.2	14 ± 3.3	6.8 ± 1.2	12 ± 3.9	4.1 ± 0.3	1.3 ± 0.5	2.9 ± 0.3
high dose	110 ± 31	88 ± 35	40 ± 4.1	85 ± 24	43 ± 13	77 ± 9.2	51 ± 11	26 ± 5.6	37 ± 9	2.1 ± 0.4

[a] x 10^6,

Discussion

This study showed that *M. anisopliae* incorporated into the potting medium of gerbera reduced western flower thrips (WFT) by up to 40%. However, when the thrips population increased to a very high level WFT control was reduced.
Different monitoring methods were used to ensure a reliable estimate of WFT populations. The absolute estimate of counting larvae and adults on the plants was time consuming, however a more accurate estimate was probably obtained of the populations, since changes in thrips activity did not influence these assessments. A relative estimate of the population was obtained using sticky traps, which measured adults flying above the plants. However the flight activity of WFT is influenced by light intensity and temperature (Brødsgaard, 1991b), and in week 7 the number of WFT was disproportionately low compared to the other weeks (Fig. 1), and this may have been due to less flight activity. However, the relative estimate method is less time consuming and often results in the correct proportion between populations in the different treatments, e.g. Robb (1989) found a good correlation between the number of WFT on sticky traps and in flowers.
Monitoring with blue sticky traps and counting the larvae and adults in flowers gave similar results in weeks 7 and 8 where the WFT populations were lower on *M. anisopliae*-treated plants than on untreated plants. The WFT populations on plants with a high *M. anisopliae* dose were similar to those on untreated plants when population assessment was done using blue sticky traps (Fig. 1).

Reinecke *et al.* (1990) found that 1 g Bio 1020 (a product based on *M. anisopliae* mycelium) per litre potting medium generally gave better control of *O. sulcatus* than 0.5 g per litre, however in some cultures e.g. chrysanthemum, control was similar for the two treatments. Verkleij *et al.* (1993) also failed to find a correlation between the amount of *M. anisopliae* and the control of pea weevil *Sitona lenities*. However, Rath (1992) reported a correlation between doses of *M. anisopliae* from 10^1 to 10^7 conidia g^{-1} sand-peat mix and the LT_{50} values against the redheaded cockchafer *Adoryphorus couloni* in laboratory experiments. These negative correlations might have been due to an uneven distribution of *M. anisopliae* conidia in the soil, since potting medium as peat often contain many heterogenous particles, whereas sand is more homogenous and should provide a more uniform conidia distribution.
In this study the number of *M. anisopliae* conidia varied between replicates as well as between sampling times. This was probably due to uneven distribution of the conidia (Table 2).

The number of *M. anisopliae* conidia after 9 weeks had declined by a factor of 10 - 50 compared to the initial inoculum incorporated into the potting medium, and this most likely reduced the effect of *M. anisopliae* on WFT populations. The decline of *M. anisopliae* conidia

was unexpected since preliminary experiments had shown an increase of inoculum in sphagnum after 6 - 8 weeks at 25°C (Vestergaard, unpubl.). The decline in the number of *M. anisopliae* in the potting medium might be attributable to the activity of microorganisms in combination with abiotic environmental factors as for example high temperature (Walstad *et al.*, 1970). However soil might be expected to provide conidia more protection to dehydration as compared to foliar applications (Gaugler *et al.*, 1989). Furthermore water content can also affects conidial survival, Li and Holdom (1993) found that the survival of *M. anisopliae* conidia declined more rapidly in wet soils than in moderately dry soils. In this experiment the soil was near saturation most of the time and this may have negatively influenced the survival of *M. anisopliae* conidia.
The decline in the number of conidia may also have been because fungistatic roots exudate from gerbera plants. Zimmermann (1984) found reduced control on cyclamen compared to other plant species and Moorhouse *et al.* (1993) found that the control of *O. sulcatus* was highly dependent on the host plant species, with mortalities ranging from 20 - 60% depending on the plant species.

Thrips populations increased rapidly in the above average summer temperature, which meant that the duration of the pupal stage was reduced allowing less time for the fungus to infect the thrips. The growth of *M. anisopliae* might also have been affected of the high temperature, as temperatures of about 30°C can reduce the growth of *M. anisopliae* 275 which has an optimal growth temperature range of 23 - 25°C *in vitro* (Moorhouse, 1990, Vestergaard, 1995). Some thrips were observed to pupate on the plant and therefore escaped infection by *M. anisopliae*. However, only a small proportion of the total population failed to pupate in the soil. Furthermore no natural predation of thrips was observed.

M. anisopliae could probably not be used as a sole control agent of *F. occidentalis* in glasshouses. However when the fungus is applied as a supplement to existing biological controls or used in integrated pest management programs, it might be possible to keep *F. occidentalis* populations on floricultural crops under the very low damage threshold.

Acknowledgements

We thank M. Gammelgaard for technical assistance and Chr. Hansen BioSystems and the Danish Industry Ministry for the financially support.

References

BRYAN, D.E. & SMITH, R.F. (1956) The *Frankliniella occidentalis* (Pergande) complex in California (Thrysanoptera: Thripidae). *University of California Publication; Entomology* **10**, 359-410.

BRØDSGAARD, H.F. (1991a) Bionomics of thrips (Thysanoptera: Thripidae) in relation to their control in Danish glasshouse crops, Ph.D. thesis, University of Copenhagen.

BRØDSGAARD, H. F. (1991b) Insekticidresistens hos saintpauliatrips (*Frankliniella occidentalis*). Dansk Planteværnskonference 1991, sygdomme og skadedyr, 25-32.

BULLARD, G., PULSFORD, D. & RATH, A.C. (1993) BioGreen - A new *Metarhizium anisopliae* product for the control of pasture scarabs in Australia. In *program and abstracts 26th Annual Meeting Society for Invertebrate Pathology*, Asheville, NC, USA. p. 38.

DOMSCH, K.H., GRAMS, W. & ANDERSON, T.-H. (1980). Compendium of soil fungi, Vol. 1. Academic press, London.

FERRON, P. (1981) Pest control by the fungi *Beauveria* and *Metarhizium*. In *Microbial Control of Pests and Plant Diseases*, ed. Burges, H.D. pp. 465-482. Academic Press, London.

GAUGLER, R., COSTA, S.D. & LASHOMB, J. (1989) Stability and efficacy of *Beauveria bassiana* soil inoculations. *Environment Entomology* **18**, 412-417.

GOTTWALD, T.R. & TEDDERS, W.L. (1984) Colonization, transmission, and longevity of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes) on pecan weevil larvae (Coleoptera: Curculionidae) in the soil. *Environmental Entomology* **13**, 557-560.

HALL, R.A. (1976) A bioassay of the pathogenicity of *Verticillium lecanii* conidiospores on the aphid, *Macrosiphoniella saniborni*. *Journal of Invertebrate Pathology* **27**, 41-48.

LI, D.P. & HOLDOM, D.G. (1993) Effect of soil matric potential on sporulation and conidial survival of Metarhizium anisopliae (Deuteromycotina: Hyphomycetes). *Journal of Invertebrate Pathology* **62**, 273-277.

MARCHOUX, G., GÉBRÉ-SELASSIE, K. & VILLEVIEILLE, M. (1991) Detection of tomato spotted wilt virus and transmission by *Frankliniella occidentalis* in France. *Plant Pathology* **40**, 347-351.

MILLER, D.W. (1994) Formulation and application of entomopathogenic fungi. *Proceedings of the Fifth International Colloquium of Invertebrate Pathology and Microbial control,* Society for Invertebrate Pathology, Montpellier, France, p. 315.

MOORHOUSE, E.R. (1990) The potential of the entomogenous fungus *Metarhizium anisopliae* as a microbial control agent of the black vine weevil *Otiorhynchus sulcatus*. Ph. D. thesis. University of Bath, UK.

MOORHOUSE, E.R., GILLESPIE, A.T. & CHARNLEY, A.K. (1993) Laboratory selection of *Metarhizium* spp. isolates for control of vine weevil larvae *Otiorhynchus sulcatus*. *Journal of Invertebrate Pathology* **62**, 15-21.

RATH, A.C. (1992) *Metarhizium anisopliae* for control for the Tasmanian pasture scarab *Adoryphorus couloni*. In *Use of pathogens on Scrab pest management*, eds. Jackson, T.A. & Glare, T. R. pp. 217-228. Intercept, Andover.

RAVENSBERG, W.J., DISSEVELT, M., ALTENA, K. & SIMONSE, M.P. (1992) Developments in the integrated control of *Frankliniella occidentalis* in capsicum and cucumber. *Bulletin OEPP/EPPO* **22**, 387-396.

REINECKE, P., ANDERSCH, W., STENZEL, K. & HARTWIG, J. (1990) Bio 1020, A new microbial insecticide for use in horticultural crops. *Brighton Crop Protection Conference, Pest and diseases*, 49-54.

ROBB, K. L. (1989) Analysis of *Frankliniella occidentalis* (Pergande) as a pest of floricultural crops in California greenhouses. Ph. D. Thesis, University of California Riverside.

TAVELLA, L., ARZONE, A. & ALMA, A. (1991) Researches on *Orius laevigatus*, a predator of *Frankliniella occidentalis* in greenhouses. *Bulletin. SROP*, **14/5**, 65-72.

VERKLEIJ, F.N., AMELSVOORT, van, P.A.M. & SMIDTS, P.H. (1992) Control of the pea weevil (Sitona lineatus L.) (Coleoptera: Curculionidae) by the entomopathogenic fungus *Metarhizium anisopliae* in fiels beans. *Journal of Applied Entomology* **113**, 183-193.

VESTERGAARD, S. (1995) Microbial control of the western flower thrips *Franklniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in glasshouses using entomopathogenic fungi. Ph.D. thesis. The Royal Veterinary and Agricultural University, Denmark.

VESTERGAARD, S., GILLESPIE, A.T., BUTT. T.M.., SCHREITER, G. & EILENBERG, J. (1995) Pathogenicity of the hyphomycete fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis*. *Biocontrol Science and Technology* **5**, 185-192.

WALSTAD, J.D, ANDERSON, R.F. & STAMBAUGH, W.J. (1970) Effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). *Journal of Invertebrate Pathology* **16**, 221-226.

YUDIN, L.S., CHO, J.J. & MITCHELL, W.C. (1986). Host range of Western FlowerThrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), with special reference to *Leucaena glauca*. *Environmental Entomology* **15**, 1292-1293.

ZIMMERMANN, G. (1984) Weitere versuche mit *Metarhizium anisopliae* (Fungi imperfecti, Moniliales) zur bekämpfung des gefurchten dickmaulrüsslers, *Otiorhynchus sulcatus* F., an topfpflanzen im gewächshaus. *Nachrichtenblatten Deutche Pflanzenschtzdienst* **36**, 55-59.

ZIMMERMANN, G. (1993) The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. *Pesticide Science* **37**, 375-379.

EFFECT OF ENTOMOPATHOGENIC FUNGI ON ENTOMOPHAGOUS ARTHROPODS

V.A. Pavlyushin

All Russian Institute for Plant Protection,
3 Podbelsky shosse,
St.-Petersburg, Pushkin, 1
89620 Russia

Summary

The Entomopathogenic fungi *Verticillium lecanii, Beauveria bassiana* and *Paecilomyces fumoso-roseus* have an entomocidal effect on *Chrysopa carnea* and *Ch. sinica* larvae and on *Cycloneda limbifer* (Coccinellidae) under experimental conditions. Larval mortality depends on the infection dosage. At a spore titre of 5 and 25 million/ml *Chrysopa* mortality was 4% and at 100 million/ml mortality levels reached 28%. *C. limbifer* larvae proved to be more sensitive to muscardine fungi. Late pathological effects of fungi on *Chrysopa* larvae, that survived a single treatment with an inoculum, were revealed. Mathematical models of pathogenic effects of fungion *Chrysopa* are proposed.

Introduction

The development of modern systems of biological plant protection and the necessity to take into account a wide influence of microbiological preparations on the elemnents of a biocoenosis put forward the problem of detailed studies of interrelations between entomopathogenic microorganisms and useful entomophagous insects. In this connection, it's very important to select test systems to assess effects and posteffects of entomophathogen applications. The pathogenicity of *V. lecanii* to honey bees (Balazy, 1972) and *Encarsia* is known (Ekbom, 1979), as well as the susceptibility of coccinelids to *B. bassiana*. Susdalskaya (1956) reported on the resistance of *Ch. ventralis* larvae to the causal agent of white muscardinosis of insects.

Investigations have been conducted in Russia proving the effectiveness of *Ch. carnea, Ch. sinica* and *C. limbifer* to control aphids in glasshouse vegetable crops. Another component of biological control in glasshouse cucumbers and tomatoes is the application of biological preparations (Verticillin, Boverin and others) based on the entomopathogenic fungi. In this connection, there exists a necessity to optimize the complex of combined applications of aphidophages and biopreparations.

Materials and Methods

Strains of entomopathogenic fungi (*V. lecanii* - 6-1; *B. bassiana* - K; *P. fumoso-roseus* - 114) were cultivated on the substrate of the following content (g/1): KH_2PO_4 - 2,0; $(NH_4)_2SO_4$ - 1,0; $MgSO_4$ - 1,0; glucose - 20,0; yeast extract - 1,0; peptone - 1-,0; agar - 20,0. As inoculum, spore aqueous suspensions at $5x10^6$ to $4x10^8$ spores/ml were used. Strain virulence was assessed on the larvae of *Ch.*

carnea (Lazarevskaja population), *Ch. sinica* (Ussurijsk population) and *C. limbifer* (Cuba, fam. *Coccinellidae*). Besides virulence, posteffects of fungi on *Chrysopa* during 5 generations after treatment were assessed. The following aspect were taken into consideration: cocoon characteristics, percentage of emerged males and females, fertility and voracity of larvae.

Mathematical models of the pathogenic action were constructed on the basis of the multiple regression analysis methods.

Results and Discussion

All studied fungi demonstrated an entomopathogenic effect on the experimentally inoculated test insects. At the inoculum titre of 10^8 spores/ml mortality of *Chrysopa* larvae varied from 27% to 38%. Insects died mainly on the second day after inoculation. *P. fumoso-roseus* demonstrated a very height entomocidical activity. Dependence of larvae mortality of *Chrysopa* and *Cycloneda* on the infection load was shown (Table 1).

Table 1. Dependence of *Ch. carneae* larvae mortality (L2) on the infection load in case of an experimental inoculation with entomopathogenic fungi.

Spore titre in the inoculum, mln/ml	Percentage larval mortality 10 days after inoculation	
	V. lecanii	*P. fumoso-roseus*
5	3,3	0
25	3,3	11,7
50	16,7	16,6
100	28,3	29,3
400	58,3	-
Control (H_2O+Triton X 100)	0	0

C. limbifer proved to be more susceptible to muscardine fungi. A higher spore titre resulted in a higher mortality of larvae: 5 mln/ml - 5%, 50 mln/ml - 30%, 100 mln/ml - 50% mortality of *Cycloneda.* Mathematical models of *Chrysopa* larvae mortality were constructed. For *Chrysopa carnea* larval infestation with *V. lecanii* (titre 50 mln/ml) the equation is:

$$Y = 19{,}9-0{,}151d+0{,}006313d^2-0{,}000026d^3+0{,}13;$$

where:
y - % mortality 10 days after treatment
d - infection load in mln/ml

A full parasitic cycle of fungal development was observed in the infected insects: conidial germs on the cuticle, isolated blastospores in the body cavity, primary mycelium in the haemolymph and well-developed mycelium in the body cavity.

intensive spore formation on cadavers.

Late effects of exposure to entomopathogenic fungi on *Chrysopa carnea* are demonstrated in Table 2.

Table 2. Late effects of entomopathogenic fungi on *Chrysopa carnea*.

Pathogen	% Imago emergence	Pertility of 1 female for 30 days	Voracity of larvae (number of eaten aphids)	% Cocoon formation	Weight of the cocoon mg
V. lecanii	40	23	243	55	5,5
B. bassiana	48	32	214	50	5,5
P. fumoso-roseus	49	43	228	55	5,0
Control H20+Triton X 100	92	88	257	80	10,2

Note: Titre of the inoculum - 10^8 spores/ml; Data are given on the first generation of *Chrysopa carnae* after the fungal treatment.

Toxic effects of fungi on the surviving insects can be traced untill the 5th generation. It's especially obvious in the 1st generation after inoculation. It's manifested in 2-times decrease of emerged imago, development of teratogenic forms, 2-3 times decrease of fertility and voracity.

Pathogenic post-effects are rather obviously manifested in the cocoon formation process: the number and size of cocoons is always smaller compared to the control; *Chrysopa* often dies inside such cocoons.

Conclusions

1. Aphidophages *Ch. carnea*, *Ch. sinica* and *C. limbifer* proved to be susceptible to muscardine fungi infestations under conditions of experimental inoculation at spore titers above $2,5x10^7$.
2. Post-effects of muscardine fungi on survived insects were registered, which manifested in deviations from the normal, as small number of emerged imago, low fertility and voracity, defects in cocoon and wing formation.

References

B.S. Ekbom, 1979. Investigations on the potential of a parasitic fungus *Verticillium lecanii*) for biological control of the greenhouse whitefly (*Trialeurodes vaporariorum*). Swed.J.Agr. Res. Vol. 9, N4, 129-38.

M.V. Suzdalskaya, 1956. On relations between chrysopa larvae (*Chrysopa ventralis Curt. subsp. prasina Burn.*) and white muscardine fungi. Zool. J., Vol. 35, N 10, 1585-1586 (In Russian).

SUBGROUP "FUNGI"

SOUS-GROUPE "CHAMPIGNONS"

Report of the general discussion, Thursday 1st September

Rapport de la discussion générale, jeudi 1er septembre

by Bernard PAPIEROK, Convenor of the Subgroup

par Bernard PAPIEROK, Animateur du Sous-groupe

B. PAPIEROK opened the discussion by suggesting some points which, in his opinion, appeared interested to be tackled. In the absence of any opposition, these points were discussed as follows.

B. PAPIEROK a ouvert la discussion en proposant quelques points qu'il lui paraisait intéressant d'aborder. Aucune objection n'ayant été émise, les points suivants ont été abordés.

I. Overview of the Poznan meeting

Most of the contributions were devoted either to ecology, in broad sense, of entomopathogenic fungi, or to the evaluation of these microorganisms as biological control agents against several pests, especially in greenhouses. Ecological contributions were dealing with natural occurring fungi, their possibly significant action in regulation of pest populations and their diversity. A few contributions were devoted to locust control by means of fungi, formulation and aplication aspects being taken into account. There was only one contribution on interactions between a fungus and a forest insect, whereas the use of pathogens to control insect pests in forestry was a special topic of the meeting! Only one contribution also was dealing with more fundamentals aspects (chemistry of secondary metabolites).

I. *Considérations générales sur la réunion de Poznan*

La plupart des communications (orales ou sous forme d'affiches) étaient consacrées à l'écologie, au sens large, des champignons pathogènes d'insectes ou à l'appréciation de leur potentiel d'utilisation dans la lutte biologique contre différents ravageurs, particulièrement sous cultures protégées. Les contributions dans le domaine de l'écologie concernaient les espèces fongiques naturellement actives dans les peuplements d'insectes. Quelques contributions ont porté sur les champignons dans la lutte contre les acridiens, les problèmes liés à la formulation et à l'application étant évidemment abordés. Une seule communication (orale) a été consacrée aux insectes des forêts, ceci alors qu'un des thèmes spéciaux de la réunion était justement l'utilisation des agents pathogènes dans la lutte contre cette catégorie de ravageurs. De même, une seule contribution (affiche) a concerné des aspects plus fondamentaux (analyse chimique de métabolites).

II. Going back over previous meetings

B. PAPIEROK reminded the audience of the results of the survey related to the proposition made by Siegfried KELLER in 1993, in Zurich, to take not only Deuteromycetes but also Entomophthorales into account when studying side effects of pesticides with regard to entomopathogenic fungi. The results are as follows:

A total of 52 forms were sent, from which 24 came back. The answer was yes for 22 of them, whereas 2 people did'nt given a firm opinion. 15 of the 22 positive answers were with no peculiar comment. The comments and remarks raised on the remaining 7 answers related essentially to a few basic questions (is it or not justified to separate effects on Beauveria bassiana or Metarhizium anisopliae on pesticides applied on the soil or into the soil, and effects on Entomophthorales of pesticides applied on plants? should one think of more in-depth studies devoted to entomopathogens-pesticides interactions? is it necessary or not to distinguish studies on commercial fungal preparations from those on naturally occurring fungi? are the side effects of pesticides to be done in both cases? is Erynia neoaphidis the best choice among epigaeic fungi?) and to practical considerations (choice of bioassay method, lack of standardisation in colonization of kernels by the fungus, uncertain feature of naturally occurring epizootics in the field, organism responsible for doing the tests?)

II. *Retour en arrière*

B. PAPIEROK rappelle les résultats de l'enquête menée suite à la proposition faite par Siegfried KELLER en 1993, à Zürich, de prendre en compte non seulement des Deutéromycètes mais également des Entomophthorales dans l'étude des effets secondaires des produits phytosanitaires.

Un total de 52 questionnaires ont été envoyés; 24 ont été reçus en retour. La réponse était oui pour 22 d'entre eux, tandis que 2 personnes ne s'étaient pas clairement prononcées. Sur les 22 réponses approuvant la proposition, 15 le faisaient sans commentaire particulier ni réserve. Les remarques soulevées sur les 7 autres réponses avaient essentiellement trait à des questions de fond (est-il justifié de distinguer, aux niveaux des épreuves à réaliser, d'un côté les effets sur Beauveria bassiana ou Metarhizium anisopliae des produits appliqués sur et dans le sol, de l'autre les effets sur Entomophthorale des produits appliqués sur les plantes? doit-on envisager également des recherches plus approfondies sur les interactions champignon entomopathogène-produits? n'est-il pas nécessaire de distinguer les essais à mener sur les préparations commerciales de champignon et ceux sur les espèces fongiques actives dans la nature? corrélativement, est-ce que les études des effets secondaires sont nécessaires dans les deux cas de figure? le choix de la seule espèce Erynia neoaphidis pour les champignons épigés est-il judicieux?) et à des considérations pratiques (choix de la méthode d'estimation de la virulence, problèmes d'homogénéité dans la colonisation des grains d'orge par le champignon, caractère aléatoire de la possibilité de disposer de parcelles au champ avec des épizooties naturelles, organisme effectuant les essais?).

III. Turning towards the future: the Copenhagen meeting

The next meeting of the Working Group would be held in Copenhagen, in 1997, organized by Jorgen EILENBERG and his team. The participants to the discussion all agreed in finding that workshops should be organized within the meeting, following the plenary session. Such workshops would be devoted to a special subject. The participants have indeed pointed out that they would be very interested to learn more on some basic aspects, which they are not too familiar with. In this prospect workshops would be entrusted to key speakers with teaching ability. On the other hand, these workshops should also include people directly in charge of plant protection. Some topics were put forward: fungal communities in the broad sense, relations between bioassays and field expected efficacy, application, effects of pesticides on fungi, toxins, use of molecular biology and genetics methods.

III. *Dans la perspective de la prochaine réunion de Copenhague*

La prochaine réunion du Groupe de Travail se tiendra à Copenhague en 1997; elle sera organisée par Jorgen EILENBERG et son équipe. Les participants à la discussion ont tous été favorables à ce que des ateliers (réunions spécialisées) soient organisés à la suite de la session plénière. Les participants ont en effet souligné qu'ils étaient intéressés d'améliorer leurs connaissances sur des aspects bien précis, avec lesquels ils ne sont pas toujours familiarisés. Dans une telle perspective les ateliers devraient être confiés à des spécialistes de bon niveau et capables de

faire un effort pédagogique. Par ailleurs, de tels ateliers pourraient s'ouvrir aux personnes directement impliquées dans la protection des plantes. Plusieurs sujets d'ateliers ont été suggérés: communautés fongiques, lien entre les résultats des essais biologiques et l'efficacité des applications sur le terrain, application, effets des produits phytosanitaires sur les champignons, toxines, utilisation des méthodes modernes de biologie et de génétique moléculaires.

IV. To strenghten East-West collaborations

An interesting fact that emerged from this meeting was the complementarity of research approaches by eastern and western teams. For instance, Polish entomo-mycologists are specialized in ecological studies (occurrence of fungi in natural systems, soil fungi: biodiversity and competition, effects of pollution), whereas western teams, in Denmark now or in France several years ago, are working on epidemiology of fungal diseases in pest insect populations. It is now righ time to firm up this complementarity by setting up collaborative projects. To develop an integrated approach to study entomogenous fungi as part of biological resources in sustainiable agriculture appeared indeed as a joint challenge.

IV. *Développement de collaborations est-ouest*

Un élément intéressant qui ressort de cette réunion, est la complémentarité des approches développées par les équipes d'Europe occidentale et orientale. Ainsi, les entomo-mycologistes polonais s'intéressent particulièrement à l'écologie des champignons (dans le sol; biodiversité d'antagonismes, effets de la pollution) alors que des équipes occidentales, au Danemark comme en France il y a quelques années, travaillent sur l'épidémiologie des mycoses dans les populations de ravageurs. C'est maintenant le moment de renforcer cette complémentarité par la mise en place de projets de collaboration. Dans cette perspective, l'étude des champignons entomopathogènes, considérée comme partie intégrante des ressources biologiques, apparaît véritablement comme un objectif majeur.

V. IOBC/WPRS Entomo-mycologists Directory

It is agreed that a directory form would be sent to all the scientists working on entomopathogenic fungi in Western Europe in order to make up a directory. The form should be also sent to Eastern colleagues. B. PAPIEROK would be in charge of this job.

V. *Répertoire OILB/SROP des entomo-mycologistes*

Il est convenu qu'un formulaire sera adressé à chaque personne travaillant sur les champignons pathogènes d'insectes en Europe de l'Ouest, ceci afin d'établir un répertoire. Le formulaire sera également adressé aux entomo-mycologistes de l'Europe orientale. Cette charge incombera à B. PAPIEROK.

5. Entomopathogenic Viruses

UV-INACTIVATION OF BACULOVIRUSES: THE BISEGMENTED SURVIVAL CURVE

J. HUBER AND C. LÜDCKE

Federal Biological Research Centre for Agriculture and Forestry,
Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany

The shape of survival curves of microorganisms subjected to the lethal action of chemical or physical agents has been the topic of many scientific controversies and discussions (for review see HIATT, 1964). For many pathogens it was found that survival during exposure does not follow a simple exponential curve as it would be the case for a normal single-hit inactivation mechanism. Instead, the curve is bisegmented: an initial steep decline is followed by a much shallower part. Both segments of the curve can be described quite well by two individual exponential curves. With insect pathogens, this tailing effect has been described, e.g., for *Bacillus thuringiensis* spores in the field (PINNOCK, *et al.*, 1975) or for baculoviruses exposed to natural sunlight on glass plates (JONES *et al.* 1993). So far, no conclusive explanation for this phenomenon could be given. The aim of this study was to get more information on the kinetics of the inactivation of granulosis viruses exposed to artificial UV irradiation.

Material and Methods

Virus: For the study, a suspension of the granulosis virus of the false codling moth, *Cryptophlebia leucotreta*, (CIGV) was used (FRITSCH, 1988). The virus was propagated in fourth instar larvae of the codling moth, *Cydia pomonella*, and purified by differential centrifugation.

UV irradiation: Dry deposits of the virus in petri dishes were irradiated with artificial light from four Ultra-Vitalux lamps (OSRAM). The emission spectrum of these lamps is very similar to natural sunlight. In order to study the kinetics of UV-inactivation, the irradiation was intermitted in some of the trials after 60 and 90 minutes respectively, and the virus was stored for 10 days at 4 or 22 °C, either as a dry deposit in the petri dish, or resuspended in Tris-buffer. In the later case it was plated out again in petri dishes for subsequent irradiation.

Bioassay: The activity of the virus was determined in bioassays with neonate false codling moth larvae, using a diet incorporation technique originally developed for codling moth (HUBER, 1981).

Results

The bisegmented shape of the survival curve could be confirmed also for the inactivation of the granulosis virus of the false codling moth (Fig. 1). During the first 60 minutes of irradiation the inactivation of the virus was with a half-life of 15 minutes rather fast. After this time about 95% of the virus had been inactivated. The remaining 5% were subsequently inactivated at a more than 8 times slower pace, showing a half-life of 124 minutes. For the second series of trials, where irradiation was intermitted for 10 days (Fig. 2 and 3), the half-life of the virus was calculated to be 18.1 minutes during the first 60 minutes of exposure to UV. When irradiation was continued immediately, half-life increased to 120 minutes, similarly to the results of the previous experiment. However, when irradiation was resumed after a break of 10 days, the inactivation was with an

average half-life of 19.8 and 22.7 minutes for intermission after 60 and 90 minutes, respectively, as fast as during the first part of the irradiation experiment. The conditions of storage during the 10 days seemed to have had little influence on the activity of the virus.

Discussion

Several attempts have been made to find explanations for the bisegmented shape of the survival curve. Some authors thought that a proportion of the virus population survived much longer because they were just better protected through shading by foliage, or because they were hidden in crevices of the plant's surface. This might be an explanation for the results of field trials, but, as demonstrated in the studies above, the phenomenon of the bisegmented curve also appears in laboratory experiments, were the virus is exposed on the surface of petri dishes, without any possibility of protection.

Often, it was also assumed that a part of the virus population would be more resistant to UV irradiation than the rest of the viruses. Based on this assumption, attempts have even been made to select for viruses more resistant to UV for use in viral pesticides. But also this theory is contradictory to the findings reported above. If a part of the virus population would really be more resistant, one would expect the half-life during the second phase of inactivation to be the same, regardless of whether the irradiation was continued immediately or intermitted for 10 days. Contrary to this, it was found that after the break, the viruses that had survived the first 60 or 90 minutes of irradiation were as sensitive as unirradiated virus.

This explanation also ruled out, there are still other possibilities remaining. One reason for the tailing effect of the survival curve could be that there is some mechanism of virus reactivation involved. Since this restoration of virus activity would have to occur during the irradiation process, it could be some kind of photo-reactivation. A mathematical model, based on this assumption, was capable of precisely describing the shape of the inactivation curve found in the trials (Fig. 4). However, a very similar shape of the curve would also be obtained if more than one mechanism were involved in the inactivation process (HIATT, 1964). This could be the case, when during the irradiation, the virus, or the DNA contained in the virus, would break up into two forms with different sensitivity to UV, or if, again during irradiation, some sort of protection against UV would build up in the viruses. Further studies will be necessary to test these theories and to study the kinetics of virus inactivation in more detail.

References

FRITSCH, E., 1988. Biologische Bekämpfung des Falschen Apfelwicklers, *Cryptophlebia leucotreta* (Meyrick) (Lep., Tortricidae), mit Granulosevirus. Mitt. Deut. Ges. allg. angew. Entomol. **6**: 280-3.

HIATT, C.W., 1964. Kinetics of the inactivation of viruses. Bacteriol. Rev. **28**:

HUBER, J., 1981. Apfelwickler-Granulosevirus: Produktion und Biotests. Mitt. Deut. Ges. allgem. angew. Entomol. **2**: 141-45.

JONES, K.A., MOAWAD, G., McKINLEY, D.J. & GRZYWACZ, D., 1993. The effect of natural sunlight on *Spodoptera littoralis* nuclear polyhedrosis virus. Biocontr. Scien. Technol. **3**: 189-197.

PINNOCK, D.E., BRAND, R.J., MILSTEAD, J.E. & JACKSON, K.L., 1975. Effect of tree species on the coverage and field persistence of *Bacillus thuringiensis* spores. J. Invertebr. Pathol. **25**: 209-214.

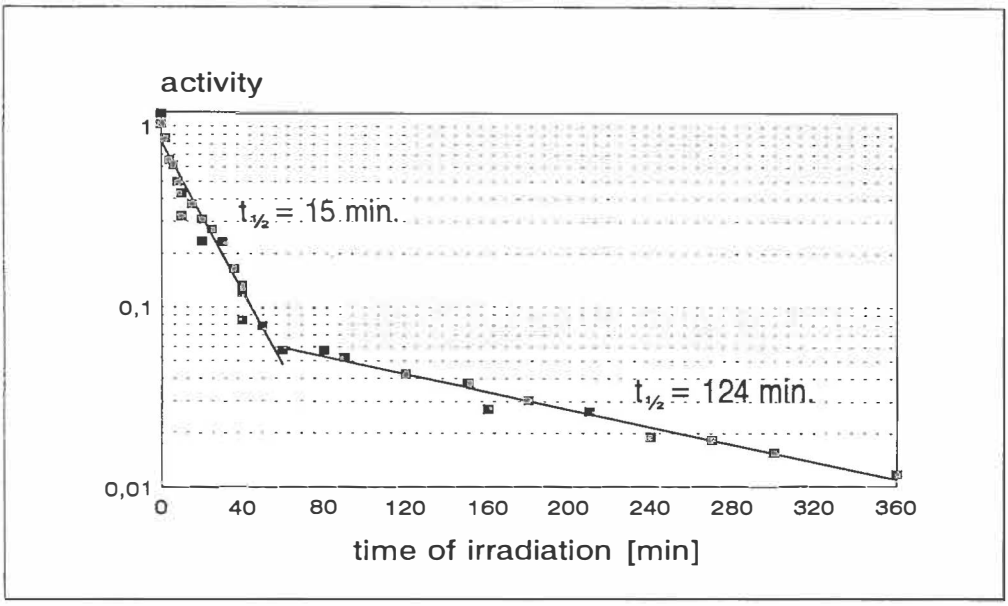


Fig. 1. Inactivation of the granulosis virus of *Cryptophlebia leucotreta* by artifical UV irradiation.

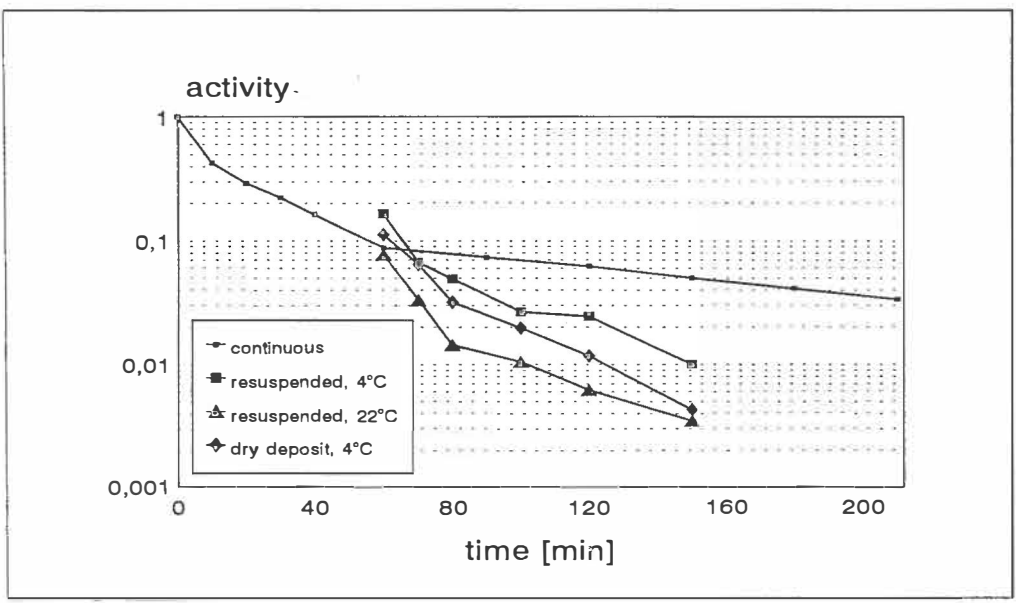


Fig. 2. Inactivation of the granulosis virus of *Cryptophlebia leucotreta* by artifical UV irradiation, intermitted for 10 days after 60 minutes.

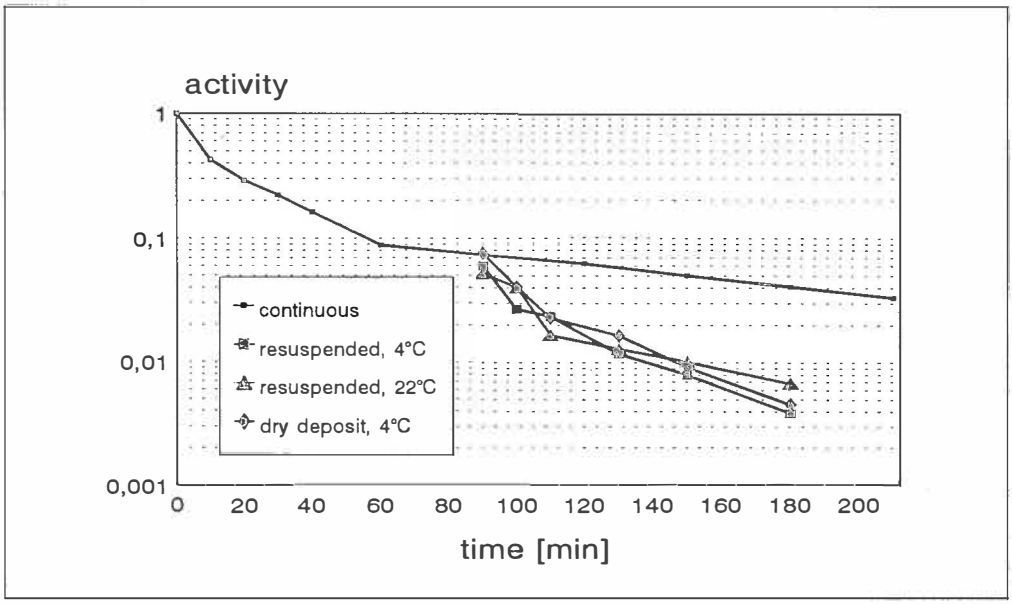


Fig. 3. Inactivation of the granulosis virus of *Cryptophlebia leucotreta* by artifical UV irradiation, intermitted for 10 days after 90 minutes.

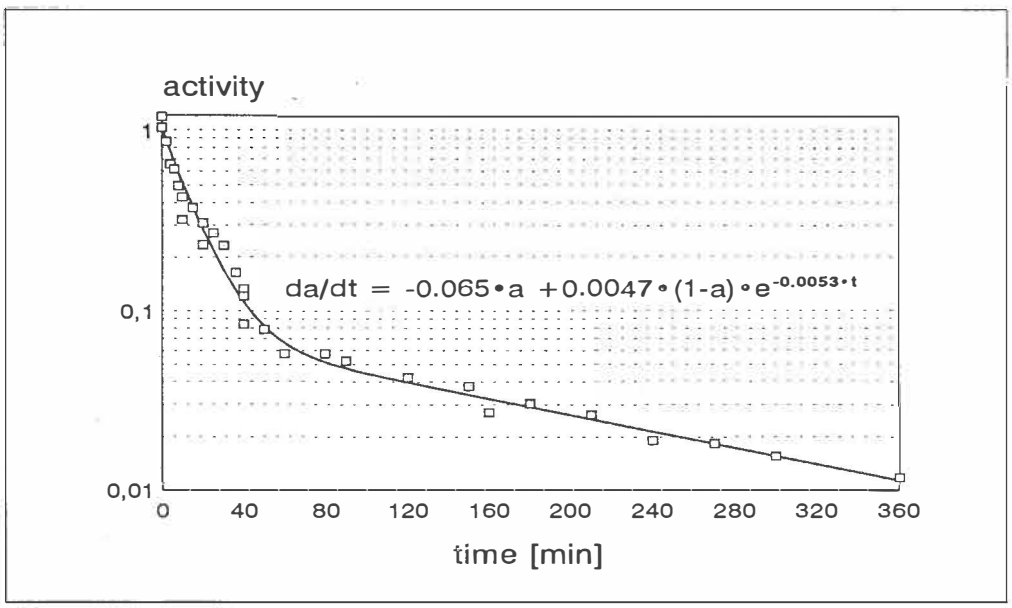


Fig. 4. Inactivation curve for a model based on photoreactivation, fitted to the data presented in Fig. 1.

MAIN CRITERIA FOR STANDARDIZATION OF QUALITY ASSESSMENT OF VIRAL PRODUCTS BASED ON BACULOVIRUSE

CIUHRII Mircea
Research Institute for Plant Protection
Bd. Ion Ionescu Dela Brad 8
71592-Bucharest, Romania

Viruses play an important role in regulating living beings populations. To this end, till now some 20 viral insecticides have been registered, designed to manage pest insect densities. All these are based on Baculoviridae, these having an unique structure and are highly specialized. Up to now baculoviruses have been exclusively isolated from invertebrate organisms, therefore these are fully harmless to man health and environment. Moreover, they are able to infect, principially, only organisms from which have been isolated. This is why WHO and FAO (1973) recommended use of baculoviruses to control plant pest insects.

Another important feature of baculoviruses is that, in most cases, are hereditary transmitted, thus acting on some biocenose components for long time.

Due to their specific structure, baculoviruses are resistant to effects of adverse environmental factors, and are able for longtims storage .

Viral products can be easily applied, with the same equipment as the chemical insecticides, or can be broadcast by some vectors, such as some entomophagous insects occurring in a given biocenose (Ciuhrii,l994).

In spite of these advantages,the viral products did not find,as yet,broad application.One of the main reasons which prevents large-scale production and use of viral insecticides is absence of a standard for assessing quality of viral products,as they differ from chemical insecticides in that they require greater responsibility from produceres,with a strict adherence to instructions for their application.Departure from these can result in failures when controlling certain pests (Ciuhrii,1988).

In 1988,a special Commission was established within IOBC-EPS in Plovdiv (Bulgaria),having as task drafting a standard for quality assessment of virus products.

In 1989 a meeting of this Commission was held in Tiraspol (Republic of Moldova) to select the main criteria usable to determine quality of viral insecticides.Standard criteria retained have been published in the IOBC-EPS Information Bulletin No.30 of 1993 (Ciuhrii and Volosciuk).A standard draft proposal has been prepared, bearing subsequent alterations which prevented registration of this standard.

At present, some additional analyses of these criteria are needed, in cooperation with IOBC-EPS scientists, before to develop common principles for virus insecticide standardization.

We are persuaded that quality of viral products cannot be merely evaluated by *the biological activity*, i.e. reaction of larvae from certain insect species to various concentrations of viral products, as these criteria are highly variable. A multitude of uncontrolled factors can affect it, in the first place the virus interaction with the host-organism in certain development stages; then, there is not possible to find standard-insects, in which a foreseen mortality following action of certain viral products can actually be achieved; therefore, this criterion cannot be standardized.

An other criterion, proposed for measurement of viral insecticides quality is concentration of product, as indicated by the number of protein formation - "polyhedra" or "granules", which can include various numbers of nucleocapsides - responsible for inducing infections. Our results (Ciuhrii, 1991) showed that in a protein formation (polyhedron), termed by us "suprapolyviriocapsides" (SPVC), and "supraviriocapsides"(SVC) for cases only one nucleocapside is included, in different insects various nucleocapside amounts can be included (*table 1*)

The highestnumber of nucleocapsides was found in SPVC isolated from *Leucoma salicis* - 2.520 + 17.2, and the lowest in SPVC isolated from *Operophtera bruceata*, Canadian population - 24+1.93, and *Heliothis armigera*, Uzbekistan population - 25+ 0.38, that means that concentration of viral insecticides should take into account the number of nucleocapsides, and the number of SPVC or SVC, which are not responsible for infection. Moreover, as sometimes nucleocapsides are lack from the protein matrix of SPVC or SVC (Ciuhrii, 1988). Therefore, we developed a special method for counting nucleocapsides included in SPVC or NPV. This needs electron microscope investigation on ultrathin sections, to determine diameter of sections (D), length of baciliform nucleocapsides (h), and the total number of nucleocapsides in a SPVC section (n). Further, the number of nucleocapsides N is determined with the formula :

$$N = \frac{D}{h} \times n$$

The number of nucleocapsides in a cm^3 with a defined titer (T) is given by the formula:

$$M = \frac{D}{h} \times n \times T$$

Thus, concentration of viral insecticides is determined by the number of nuclecapsides responsible for infection, and by protein SPVC or SVC.

Table 1

Number of nucleocapsides included in SPVC of NPV isolated from various insects species

Insect species	Population	Number of nucleocapsides included in a SPVC
Mamestra brasicae	Moldova	444 ±10.3
Mamestra oleraceae	Moldova	309 ±7.29
Heliothis armigera	Uzbekistan	25 ±0.38
Agrotis segetum	Moldova	324± 8.81
Spodoptera litoralis	America	273± 7.06
Lymantria dispar	Canada	610 ±15.08
Lymantria dispar	Moldova	1215± 15.60
Malacosoma neustria	Moldova	200± 8.28
Euproctis chrysorrhoea	Moldova	645 ±8.91
Erannis defoliaria	Moldova	110 ±562
Leucoma salicis	Moldova	2520 ±17.2
Leucoma salicis	Poland	2096 ±12.62
Iponomeuta malinellus	Moldova	314 ±6.30
Cingilia caterparia	Canada	182 ±3.02
Horisteneura fumiferana	Canada	185± 2.39
Thymelicus liniata	America	60 ±2.72
Oporophtera bruceata	Canada	24 ±1.93
Orgya leucostigma	America	137 1±3.02

In various phases of insecticide production a certain amount of nucleocapsides can be lost. This loss should be taken into acount. Likewise, during viral product storage part of nucleocapsides is destroyed. Our investigation (Ciuhrii, 1988) estabilished the speed of destruction of SPVC nucleocapsides under various storage conditions and depending on insecticide formulation. In table 2 information are given on changes in some parameters : Y (t) -amount of normal nucleocapsides present in SPVC, and C (t) -amount of destroyde nucleocapsides in a determined interval. Having these data included in table , the suitable dose of viral insecticide can be determined, as depending on periad of storage and keeping conditions. Use of this table allows to accurately adjust the rate per hectare of the product, which contains an infective quantity able to induce an artificial infection, corresponding to the initial dose of products.

Table 2.

Parameters Y (t), C (t) storage of viral insecticide Virin-ENch for 24 months

Months	-15° C		4°C		+10....15°C	
	Y %	C	Y, %	C	Y,%	C
-	100.0	1.00	100.0	1.00	100.0	1.00
1	99.7	1.00	97.4	1.03	96.8	1.03
2	99.3	1.01	94.9	1.05	93.6	1.07
3	99.0	1.01	92.5	1.08	90.6	1.10
4	98.7	1.01	90.1	1.11	87.6	1.14
5	98.4	1.02	87.8	1.14	84.8	1.18
6	98.0	1.02	85.5	1.17	82.0	1.22
7	97.6	1.02	83.3	1.20	79.4	1.23
8	97.4	1.03	81.2	1.23	76.8	1.30
9	97.1	1.03	79.1	1.26	74.3	1.35
10	96.8	1.03	77.0	1.30	71.9	1.39
11	96.4	1.04	75.0	1.33	69.6	1.44
12	96.1	1.04	73.1	1.37	67.3	1.49
13	95.8	1.04	71.2	1.40	65.1	1.54
14	95.5	1.05	69.4	1.44	63.0	1.59
15	95.2	1.05	67.6	1.48	61.0	1.46
16	94.9	1.05	65.9	1.52	59.0	1.70
17	94.5	1.06	64.2	1.56	57.1	1.75
18	94.2	1.06	62.5	1.60	55.2	1.81
19	93.9	1.06	60.9	1.64	53.4	1.87
20	93.6	1.07	59.3	1.69	51.7	1.93
21	93.3	1.07	57.8	1.73	50.0	2.00
22	93.0	1.08	56.3	1.78	48.4	2.07
23	92.7	1.08	54.9	1.82	46.8	2.14
24	92.4	1.08	53.5	1.87	45.3	2.21

So, the number of normal nucleocapsides contained by SPVC or SVC,as instrumentally evinced, is one of basic criteria which determines presence of the potential viral insecticide, able to act normally in inducing a viral epidemy.

An other important criterion required by quality standardization of viral insecticides is the identity of virus strain with the initial strain, as the danger exists during production of the viral insecticide, that another virus strain be activated, this latter having unsuitable characteristics to the given product. Identity of a strain can be assessed with certain accuracy by an ELISA-test; however, there are also some difficulties. The first is

the need to possess certain standard enzymes, then the use of materials to deproteinize baculoviruses.

We have developed a morphological method for VPN identification, based on the following criteria: occurrence of virions or polyvirions included in SPVC; frequency of polyvirions, which contain a certain number of nucleocapsides (from one to five,or even more); the total number of nucleocapsides in SPVC; presence of electron - dense evnelopes of SPVC.

These analyses are performed simultaneously with detection of presence of a certain number of nucleocapsides exhibiting normal structure, and including SPVC. Thus, quantitative and qualitative analyses are effected, using the method previously proposed (Ciuhrii, Volosciuc et al.,1991).

Criteria exposed above include only pathogen characteristics, this presenting the biologically active matter. However, there are still other peculiarities of matrix, which equally determine quality of insecticides. Firstly, the occurrence degree of extraneous microflora is determined. Then, the following parameters are measured: particle size of ingredients forming the matrix ; degree of formation of stable suspension; resistance of viral infection to sunbeams; adherence degree on leaves; degree of humidity; pH of medium; suitability of packaging and labelling; external appearance corresponding to characteristics indicated in instructions.

Analysis of parameters listed is performed following certain methods of determination, specified in the standard draft already published (Ciuhrii, Volosciuc, 1993). The recommended methods are endorsed and adapted by specialists in the given domain.

During production of viral insecticides, which are standardized in agreement with all parameters indicated above, checking being made only on pathogen concentration, as indicated by the number of normal nucleocapsides included in SPVC or SVC, identity of pathogen strain and degree of extraneous flora. The other parameters are checked only when some changes occurred in technological parameters of production.

Adoption of such a standard will raise responsibility of producer and guarantee efficiency of viral insecticides in the integrated control of pest insects.

A NEW POSSIBILITY FOR CONCENTRATION DETERMINATION OF VIRAL INSECTICIDES

CIUHRII G. Mircea
Research Institute for Plant Protection
Bd. Ion Ionescu Dela Brad 8
71592 -Bucharest, Romania

Concentration of viral insecticides plays a definite role during their utilization. Unfortunately, till now concentration of viral insecticides based on baculoviruses is not performed starting with the number of nucleocapsides, but merely with the number of protein formations (polyhedra, granules, bodies), these containing very variable numbers of nucleocapsides (Ciuhrii, 1988, 1991).

The protein formations termed by us "suprapolyviriocapsides" (SPVC) when contain several nucleocapsides, and "supraviriocapsides" (SVC), enclosing an unique nucleocapside.We think that these terms express more accurately the structure and function of these formations, which are fundamentally capsides of nucleocapsides (virions).They cannot be simply called "polyhedra" or "bodies", as these can be easily confounded with other cell structures or formations of different nature.

For instance, the SPVC NPV isoladed from *Leucoma salicis* includes on average 2.520 æ 17.2 nucleocapsides, while *Heliothis armigera* NPV contains some 25 æ 0.38 nucleocapsides in SPVC. Viral products recommended to control these pests contain approximatively the same SPVC concentration, i.e. 6×10^9 per gram of dry product. However, differences among the numbers of nucleocapsides included

in SPVC are very broad and make questionable calculations presented. Till now, no possibilities for determining the number of nucleocapsides contained by SPVC was known. We succeeded in developing such a procedure. Essentially, this performs the following steps: the viral product is fixed for effecting electron microscope investigations; ultrathin section are prepared; diameters of sections through SPVC (di) can be established directly on the fluorescent screen of an electron microscope, or on photographs taken later; the number of nucleocapsides (ni) is counted in these sections (m).

Diameter of SPVC ultrathin sections is given by the formula:

$$d = \frac{l}{m} \sum_{i=l}^{m} d_i \qquad (1)$$

and the average number of nucleocapsides in a section:

$$n = \frac{l}{m} \sum_{i=l}^{m} n_i \qquad (2)$$

The diameter (D) of an average SPVC is determined, "d" being the average factor of diameter of SPVC sections.In this case SPVC can be compared with a sphere.The diameter D can be equalled with a cylinder,having "d" as diameter,and "D" as its heigth :

$$\frac{\pi D^3}{6} = \frac{\pi d^2}{4} D \qquad (3)$$

or:

$$D = 1.5\ d \qquad (4)$$

Thus,one can consider that any section of this cylinder can contain a certain number of nucleocapsides (n).

The average length (h) of a nucleocapside is determined.To simplify the procedure,the nucleocapside is equalled with a segment of line "l".As the nucleocapsides

are randomly distributed in the SPVC protein matrix,one can consider that heads of A,B nucleocapsides (fig.1) occur with the same probability everywhere in a sphere having "l"

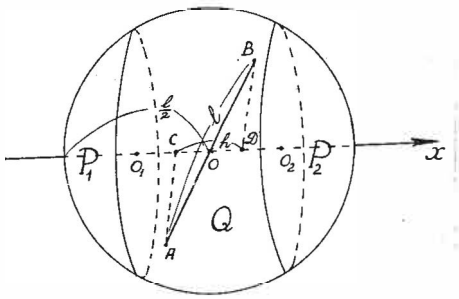


Fig.1.

as diameter,and its centre being located in a certain point "O".Let us consider that direction of axis "x" passing through the centre of the baciliform nucleocapside (point "O") coincides with direction of cylinder axis.Then,the projection of segment "A,B" on "x" axis (segment CD) is equivalent to "h" and will be the distance within the cylinder.The average distance "h" can be determined as follows:the volume of sphere is divided into 3 parts :two are equal,symmetrical to the centre "O" of curves of superficial segments of the sphere "P1"and "P2",and of central surface "Q".Lets calculate the distance between O1 and O2 to the two circles segments the sphere,so that P1+P2 = Q.

$$P_1 = P_2 = 2\pi\frac{l}{2}\left(\frac{l}{2} - OO_1\right) = \pi l\left(\frac{l}{2} - OO_1\right);$$

$$Q = 4\pi\left(\frac{l}{2}\right)^2 - 2P_1 = \pi 1 - 2\pi 1\left(\frac{l}{2} - OO_1\right) = 2\pi l.OO_1;$$

$$2\pi l\left(\frac{l}{2} - OO_1\right) = 2\pi l.OO_1;\ OO1 = \frac{l}{4};$$

$$O_1O_2 - 2OO_1 = \frac{1}{2} \qquad (5)$$

Thus,projections of nucleocapsides,whose ends occur on the surface Q,have lengths $h < O_1O_2$,while that of nucleocapsides occurring on the surfaces P1 and P2 is the length $h > O_1O_2$. Having in view that nucleocapsides are cylindrical with diameter do,we can reach the value:

$$h = \frac{d_0 + l}{2} \qquad (6)$$

The number of nucleocapsides included in the volume D shall be:

$$\frac{D}{h} \qquad (7)$$

and the number of nucleocapsides N included in one SPVC will be thus determined :

$$N = \frac{D}{h} \cdot n \qquad (8)$$

The number of nucleocapsides M in one cm^3 with a certain T titre can be determined with the formula:

$$M = \frac{D}{h} \cdot n \cdot T$$

According to the formulae (4) and (6) we will obtain :

$$M = \frac{\sqrt{6}\, d\, n\, T}{d_o + l}$$

The absolute error of value "M" is determined with the formula :

$$\Delta \max (M) = \frac{3 \cdot 2 \cdot 45 \cdot d \cdot n \cdot T}{d_o + l} \frac{s(d)}{d} + \frac{s(n)}{n} + \frac{s(d_o)+s(l)}{d_o + l} + 0.0002$$

where s(d), s(n), s(do), s(l), s(T) are the variable sizes of values d, n, do, l, T; and $\sqrt{6} \approx 2.45$.(linear values of nucleocapsides in one ml of suspension).

In order to prepare a solution from a viral product with a certain concentration (M),it is necessary to firstly determine the values d, n, do, l, and then titre (T),using the formula:

$$T = \frac{M(d_o + l)}{\sqrt{6} \cdot d\, n}$$

Thus, firstly, the average number of nucleocapsides contained by one SPVC is determined, then the titre is calculated from the number of nucleocapsides and not of SPVC, which are not responsible for inducing infections.To this end the concentration of viral suspension,as established from SPVC concentration is multiplied by the average number of nucleocapsides contained by SPVC.Thus, for instance, if the concentration of the viral product Virin - ENch is 6×10^9 SPVC per gram,this value is multiplied by the number of nucleocapsides included in SPVC, which is 1.215+-15.6 for *Lymantria dispar*, population from Moldova.

So, the concentration will be:

$$6 \cdot 10^9 \cdot 1.215 = 15 \cdot 10^{12} = 1.5 \cdot 10^{13}$$

To determine viral concentrations of a granular virus (GV) generating SVC whose size cannot be practically counted with a conventional microscope, measuremed can be effected in electron microscopy.Therefore,the viral suspension is contrasted with 2% uranyl acetate solution for 1 min and is analysed on the fluorescent screen of this microscope.Analysis is carried out in a 0.001 ml suspension pipetted on grid.The SVC are counted in a sector of the screen equal to 90 x 100 mm, using the formula: $T = 5.887 \cdot 10^6 \cdot a \cdot k$, where T = titre of viral suspension (SVC in one ml or g); a - number of SVP on the screen or photographic film; k = degree of dilution of the viral suspension.

Thus, within 15 min. SVC in 40 - 60 fields can be determined, then, using the above formula, concentration of viral suspension is accurately measured. This procedure is by far simpler and more precise than all those known till now.

Accurate determination of viral concentration from their number of nucleocapsides responsible for inducing infections will warrant achievement of the desired results.

MORPHOLOGICAL AND BIOLOGICAL CHARACTERIZATION OF A SPANISH STRAIN OF THE *LEUCOMA SALICIS* NUCLEAR POLYHEDROSIS VIRUS

P.B. MARACAJÁ, E. VARGAS-OSUNA, H.K. ALDEBIS
and C. SANTIAGO-ALVAREZ

Cátedra de Entomología Agrícola y Forestal
Departamento de Ciencias y Recursos Agrícolas y Forestales
E.T.S.I.A.M. Universidad de Córdoba. Apartado 3048.
14080 Córdoba, España.

Summary

A nuclear polyhedrosis virus has been isolated from *Leucoma salicis* (Lepidoptera, Lymantriidae) for the second time, in Northern Spain. The polyhedra measuring about 1.67 µm in diameter, include multiply-enveloped nucleocapsids. This LsMNPV isolate showed cross-infectivity to the noctuid *Discestra trifolii* and to the arctiid *Ocnogyna baetica*.

1. Introduction

The larvae of the satin moth, *Leucoma salicis*, frequently cause defoliations in poplar stands in North-West Spain. Heavy infestations result in less vigorous trees, reduced wood yield and finally death of some trees (Romanyk and Cadahia, 1992).

The control of the pest could be achieved by means of broad-spectrum chemical insecticides, but environmental reasons urge the development of new strategies. One of these strategies could involve the use of entomopathogens among which the most promising are baculoviruses whose presence in *L. salicis* populations have been early reported from Spain (Rubio-Huertos and Templado, 1958) and elsewhere (Lameris *et al.*, 1985; Ziemnicka, 1981).

During an outbreak in July 1992 in poplar plantations, in the Province of León, an epizootic caused by a nuclear polyhedrosis virus was detected. In this paper we present data on the morphology and biological activity of this second isolate of the NPV of *L. salicis* in Spain.

2. Materials and Methods

Diseased *L. salicis* larvae were collected, in poplar plantations, at Villarejo de Orbigo and Villar del Yermo (León) in July, 1992. Dead larvae were homogenized in distilled water using a glass tissue grinder. The homogenate was filtered twice through two layers of cheesecloth. The filtrate was centrifuged through a layer of 30 % (w/w) sucrose at 10,000 rpm for 15 min in a Beckman T-19 rotor. The pellet was resuspended in distilled water and it was then layered onto a two-step sucrose gradient

of 10 ml 42 % (w/w) and 15 ml 62 % (w/w) sucrose. After a centrifugation in a SW28 Beckman rotor at 25,000 rpm for 60 min, the polyhedra were collected at the interface between 42 % and 62% sucrose and washed twice in distilled water by centrifugation at 15,000 rpm for 10 min. The final pellet of purified polyhedra was resuspended in double-distilled water and stored at 4°C. The concentration of polyhedra was determined by the Malassez haemocytometer.

Polyhedra in 1 ml of the above suspension were peleted at 14,000 rpm for 10 min in an Eppendorf centrifuge and then fixed at 4°C in a cacodilate-buffered (pH 7.0) 2.5 % glutaraldehyde for 24 hours. The pellet was washed three times at 4°C in cacodilate 0.1 M (pH 7.0). The polyhedra were postfixed in cold cacodilate-buffered (pH 7.0) 1% osmium tetroxide for 2 hours. After dehydration through a graded series of ethanol, the pellet was embedded in epon and thin sectioned with a glass knife on an LKB NOVA ultramicrotome. The thin sections were stained with a 1% aqueous solution of uranyl acetate for 20 min and then with a saturated lead citrate solution for 10 min. Sections were examined with a Phillips CM-10 electron microscope operating at 80 kV. Polyhedra and virions were measured using the computer programme IMAGO developed by Electron Microscopy Service Unit of the University of Córdoba.

Since other species belonging to the family Lymantriidae were not available in the laboratory, species from Noctuidae and Arctiidae, whose stock colonies are maintained at insectary conditions on artificial diet (Santiago-Alvarez, 1977), were used in these tests. Newly moulted third instar larvae were fed for 24 hours with alfalfa leaves which had been surface contaminated with a virus polyhedra suspension. Tissues of dying larvae, and all larvae living at the end of the experiment, were examined with a phase contrast microscope for the presence of polyhedra.

3. Results and Discussion

The polyhedra shape, from electron micrographs, appeared as irregular hexagons and pentagons, measuring between 1.04 to 2.45 µm in diameter with a mean value of 1.67 ± 0.32 µm . The polyhedra of this Spanish isolate appear smaller than those of the earlier one, isolated 36 years ago, from a *L. salicis* population around Madrid (Rubio-Huertos and Templado, 1958).

Thin-sections of the polyhedra revealed that this isolate, like the former one (Rubio-Huertos and Templado, 1958), is a NPV of the multiply-enveloped type with bundles of up to 7 nucleocapsids per envelope. Our observations suggest that the endemic *L. salicis* NPV in Spain is a multiply-enveloped NPV like isolates from other geographical origins (Lameris et al. 1985; Ziemnicka, 1981).

The nucleocapsids, that measured 32.25 nm in diameter and 247.00 nm in length, are smaller than those of a Polish isolate (Ziemnicka, 1981). As these differences do not allow discrimination between the Spanish and Polish isolates, DNA

analysis techniques are required to characterize each virus isolate.

The biological activity was determined by cross-infectivity tests using larvae of 18 lepidopteran species, whose results can be seen on Table 1. The LsMNPV was cross-infective to the noctuid *Discestra trifolii* and to the arctiid *Ocnogyna baetica*. These two hosts extend the LsMNPV host range to lepidopteran species other than lymantriids (Skatulla, 1985). On the other hand, from laboratory tests, the insecticidal activity of the LsMNPV against *O. baetica* larvae was comparable to that of the *O. baetica* granulosis virus (Maracajá *et al.*, in press).

Table 1. Cross-infectivity tests of **LsNPV** on various lepidopteran species.

FAMILY	SUBFAMILY	SPECIES	
NOCTUIDAE	Amphipyrinae	*Athetis hospes*	-
		Hoplodrina ambigua	-
		Sesamia nonagrioides	-
		Spodoptera cilium	-
		Spodoptera exigua	-
		Spodoptera littoralis	-
	Noctuinae	*Agrotis ipsilon*	-
		Agrotis segetum	-
		Ochropleura leucogaster	-
		Peridroma saucia	-
	Plusiinae	*Chrysodeixis chalcites*	-
	Hadeninae	*Discestra trifolii*	+
		Mamestra brassicae	-
		Mythimna loreyi	-
		Mythimna vitellina	-
		Mythimna zeae	-
	Heliothinae	*Helicoverpa armigera*	-
ARCTIIDAE		*Ocnogyna baetica*	+

Acknowledgements

This research was supported by the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) grant AGR90-0445.

4. References

LAMERIS, A.M.C., ZIEMNICKA, J., PETERS, D. GRIJPMA, P. & VLAK, J. M., 1985. Potential of baculoviuruses for control of the satin moth *Leucoma salicis* L. (Lepidoptera: Lymantriidae). Med. Fac. Landbouww. Rijksuniv. Gent. **50/2a**: 431-439.

MARACAJÁ, P.B.; VARGAS-OSUNA, E.; ALDEBIS, H.K. & SANTIAGO-ALVAREZ, C. Poder insecticida del virus de la poliedrosis nuclear (VPN) de *Leucoma salicis* (Lepidoptera: Lymantriidae) sobre larvas de *Ocnogyna baetica* (Lepidoptera: Arctiidae). Sanidad Vegetal Plagas (in press).

ROMANYK, N. & CADAHIA. D., 1992. Plagas de insectos en las masas forestales españolas. Colección técnica M.A.P.A. Madrid. 272 pp.

RUBIO-HUERTOS, M. & TEMPLADO, J., 1958. Un virus poliédrico en *Stilpnotia salicis* (L.) (Lepidoptera, Lymantriidae). Microbiol. españ. **11**: 93-98.

SANTIAGO-ALVAREZ, C., 1977. Virus de insectos: Multiplicación, aislamiento y bioensayo de *Baculovirus*. Serie Universitaria 43. Fundación Juan March. 59 pp.

SKATULLA, U., 1985. Untersuchungen zur Wirkung eines Kernpolyedervirus aus *Leucoma salicis* L. (Lep., Lymantriidae) auf einege Lymantriiden-Arten. Anz. Schädlingsk. Pflanzensch. Umweltsch. **58**: 41-47.

ZIEMNICKA, J., 1981. Studies on nuclear and cytoplasmic polyhedrosis viruses of the satin moth (*Stilpnotia salicis* L.) (Lepidoptera, Lymantriidae). Prace Naukowe Instytutu Ochrony Roslin. **23**: 75-142.

THE PERSPECTIVE SELECTION OF HIGH VIRULENCE STRAINS OF THE ENTOMOPATHOGENIC VIRUSES

Voloshchuc L.T., Popushoy I.S.
Institute for Biological Control Academy
of Science of Moldova
Dacia Bd.58, 277060 Kishinev, Moldova

Summary

Wide application of baculoviruses insecticides as agents of plant biological protection is inseparably linked with use of high effective strains. Long investigations on the selection of strains with high biological activity showed a possibility of selection work with baculoviruses. Best results are obtained in the case of multi-step selection of pathogens with infection of unusual host insects, with selection by sign LK50, LD50, LT50, with hidrodinamic indicators and others.

Introduction

Taking into consideration a timing of Nuclear Polyhedrosis Virus to concret insect species, tendention to formation of middle virulence strains of baculoviruses, on the one hand, and increasing of agriculture and forestry needs in high effective insecticides, on the other hand, the necessity of selection of the entomopathogenic viruses to the virulence is incresing (Luckow V.A., Summers M.D.,1988).

In order to organize the selectional work with baculoviruses, right understanding of the pathogenesis processes and for wide effective application of the entomopathogenic viruses preparations it is expedient to single out the next most important proprieties of the parasites: pathogenicity, virulence and agressivity (Fuxa J.R., Tanada Y.,1987).

Pathogenicity - as baculoviruses' capacity to provoke diseases, should be understood ascriterion of the species, which determines its capacity to intracell and genetical parasitic mode of life and provoke, with presence of 3 main factors, the pathological prosess.

Virulence - as quality measure of pathogenicity, shold be understood as pathogenicity of present baculoviruses for certain insect species. So far as virulence is inherent in only pathogenic organism, the intraspecies differentiation of baculoviruses shold be done on the basis of this indicator. In the conditions of constant natural selection press, the baculoviruses are represented by numerous populations that are heterogeneous by the sign of virulence. Therefore, in the technological processes of the baculoviruses insectecides' production, it is necessary to use the most virulent strains and to create conditions favourable to the virulence increasing.

Agressivity - as quantity measure of pathogenicity, defines the baculoviruses' capacity to provoke mass diseases of susceptible insect species, i.e. provoke epizootics. Agressivity depends on baculoviruses' capacity to provoke infestation by minimal quantity of infection, i.e. minimal titres, on speed of processes of deproteinization, morfphogenesis and diffusion of the parasite in infected cells and tissues, on quantity of nucleocapsides accumulated in one polihedre, on speed of diffusion of pathogene and its stability in the cconddition s of pathogenesis. It is necessary to note that, as all quality signs, agressivity can considerably vary in the dependaance on the whole row of environment factors.It gives possibility of wide manoeuvrement by abiotic

factors contributing to the increasing of baculoviruses insecticides' quality (Chukhrii M.G., Voloshchuc L.T.,1992; Groner A.,1986; Voloshchuc L.T.,1994).

Therefore, in order of more widee production and application of viruses entomopathogenic biopreparations it is expedient to select strains with high biological activity and to develop tecnological means of increasing of baculoviruses insecticdes quality (Huber J.,1986; Tanada Y.,1985).

Materials and Mehtods

Investigations were made with laboratory cultures ***Mamestra brassicae L.*** and ***Helicoverpa armigera Hbn.***, which kept on artificial nutrient medium at temperature 23±1°C and 25±1°C, respectively. For infectation of 2-3 instar caterpillars ofinsects it was used a dosed rearing by virus suspension with titre 100 to 1 million polyhedres. Registration of results was being made daily since 3-th day after infection.

Biological activity of isolates was being defined by Abbot formula with accounting also deth degree of caterpillars in the control, as well.

For identification of baculoviruses and determination of morphological condition of pathogenes was used the method of Chukhrii M., Mencher E.(1983).

Baculoviruses fraction with different hidrodinamics indicators was obtained in saccharose density gradient (10 to 60%).

Biological testing of species, strains, isolates and fractions of baculoviruses was made in conformity with method Gar K.(1963), and mathematical treatment of results was accomphished by the method ex Dospekhov B.A.(1979).

Results and discussions

Obtained results show that specificity of baculoviruses have a relative character which is confirmed by infection of Nuclear Polyedrosis ***Mamestra brassicae*** not only ***Mamestra brassicae***, but also ***Helicoverpa armigera***. Infectation of cotton moth caterpillars by nonspecific virus is linked with original change of biological activity of the virus. In the first passage LD50 of alien virus is as mutch as 58 times LD50 of its own pathogene. In second passage it was observed a stabilization of morphogenesis processes and considerable increasing of biological activity. On electronogramms it is noted a valuable polyhedres NPV ***M.brassicae*** formation that indicates to its reproduction in nonspecific cell (Fuxa J.R.,1988; Voloshchuc L.T.,1993).

Nonspecific inffectation by baculoviruses provides, besides increasing of biolofical activity, decreasing of letal time. So LT50 of NPV ***H.armigera*** is 7.4 days, of NPV ***M.brassicae*** is 7.39 days.In first passage this indicator increases to 10.58 days, in subsequently decreasing gradually and in third and forth passages it constitute 5.65 and 5.39 days, respectively.

Formation of fraction by the way of differentional centrifugation in saccharose density gradient showed that middle fraction of polyhedres provide the highest mortality of cotton moth caterpillars with use of virus suspension with titre of 100 000 polyhedres/ml (90%). Heavy and light fraction NPV ***H.armigera*** are characterized by less biological activity (44 and 52%, respectively).

Conclusions

1.In nature evolution processes, the baculoviruses proceed apparently by the way of constant increasing of specifiicity of partner mutual relation that is accompanied by gradual decreasing

of pathogen activity thus providing their farther existense in the structure of pathogeneses. For induction of proper locuses activity and expression of genes that determine the capasity to infect insect from different grups of cross-protection, it is necessary to create conditions favourable for realization of their genetical information. There is the biopolymer composition of cell which should be attacked by baculoviruses infection activity, among such cconditions. By the way of artificial multiple passages, baculoviruses in cells and tissues regard to close femiliar (on the level of family) species, it become real the seelection of viruses for increasing of virulence and agressivity.

2.Increasing of biological activity and reduction of letal time of action of baculoviruses is possible in as a result of multy-step, in accordance with hidrodinamics indicators, instar susceptibility, pathogen going out to one caterpillar and others.

References

1.Chukhrii M.G., Voloshchuc L.F.,1992. Principles for standartization of viral insecticides. In XXY annual meeting Society for invertebrate pathology. Heidelberg, 1992, P.169.

2.Groner A.,1986. Specificity and safety of baculoviruses. In R.R.Granados and B.A.Federici. The Biology of Baculoviruses. Vol.1. P.177-202. CRC Press, Florida.

3.Fuxa J.R.,1988. New directions for insect control with baculoviruses. In Baker R., Dunn P. New Directions Biological Control. P.121-130.

4.Fuxa J.R., Tanada Y.,1987. Epizootology of insect disiases. New York.

5.Huber J.,1986. Use of baculoviruses in pest management programs. In R.R.Granados and B.A.Federici. The Biology of Baculoviruses. Vol.2. P.181-202.

6.Luckow V.A., Sammers M.D.,1988. Trends in the development of baculoviruses expression vectors. Biotech., 6, P.47-55.

7.Tanada Y.,1985. Asynopsis of stadies on the synergistic property of an insect baculovirus: a tribut to Edward A.Steinhaus. J.Invert.Pathol.,45, P.125-138.

8.Voloshchuc L.T., Chukhry M.G.,1993. On specifity of entomopathogenic viruses. Seliscohozeaistvenaea biologia. 3. P.76-89. (In Russian).

9.Voloshchuc L.T.,1994. Epizootological character of entomopathogenic viruses. Simposium 125 years of higher agronomic education at Cluj-Napoca. P.78. Cluj-Napoca.

BIOCHEMICAL CHARACTERIZATION OF NATURAL ISOLATES OF NUCLEAR POLYHEDROSIS VIRUS *STILPNOTIA* SALICIS L.

J.Michalik[1], E.Szolajska[1], J.Ziemnicka[2], O.Okunev[3]

[1] Inst.Biochem.& Biophys., Pawińskiego 5a, 02-106 Warsaw.
[2] Inst.Plant Protection, Miczurina 20, 60-318 Poznań.
[3] Inst. Molec.Biol.& Gen., Zabolotnogo 150, Kiev 143.

INTRODUCTION

The satin moth (Stilpnotia salicis) is a frequent insect pest on poplar and willow trees (Ziemnicka,1981; Lameris et al.,1985). Outbreaks of this pest were often controlled by using broad spectrum of chemical insecticides. The new strategy of controlling satin moth population involves the use of specific pheromones and baculoviruses. Baculovirus occuring in Stilpnotia salicis showed cross-reactivity to number of other Lymantrids. In this report we compare several SsMNPV isolates collected in five regions of southern and western Poland during ten consecutive years. Biochemical characterization of these isolates involves microscope comparison of polyhedra size and shape, restriction analysis of virus DNA and comparison of virus polypeptides.

MATERIAL AND METHODS

MATERIAL: *Stilpnotia salicis* larvae infected with multiple nuclear polyhedrosis virus (MNPV) were collected during 1971 - 1980 years on poplar trees in the second half of May in five different regions of Poland. Infected larvae were dried and stored at 4°C.

ISOLATION AND PURIFICATION OF POLYHEDRA: polyhedra were obtained from dried down larvae kept at 4°C. Larvae were homogenized in the solution of 1% SDS and filtrated through two

layers of cheesecloth. The filtrate was centrifuged at 10 000 rpm for 30 min. The pellet was suspended in TE buffer and 10 ml portions were layered onto a two-step sucrose gradient of 20 ml 50% (w/w) sucrose and 6 ml 60% (w/w). After centrifugation in SW 28 Beckman rotor at 24 000rpm for 60 min., the polyhedra were collected at the interface between 50% and 60% sucrose. Polyhedra were subsequently washed three times with distilled water and the final pellet was resuspended in water and stored at 4°C. Amount of polyhedra was calculated in standard chamber hemocytometer.

MICROSCOPE ANALYSIS OF POLYHEDRA: purified polyhedra were examined by light and electron scanning microscopy. In studies by light microscope polyhedra were stained with acid carbol-fuchsin (Evlachova and Svecova,1964). Samples for scanning microscope were critical point dried, coated with gold and photographed.

ISOLATION AND PURIFICATION OF VIRAL DNA: DNA of ss MNPV was isolated from purified polyhedra virions according to modified method of Czerepenko and Martynienko, 1985.

ANALYSIS OF VIRUS DNA WITH RESTRICTION ENDONUCLEASES: DNA concentration was estimated during electrophoresis in 0,8% agarose; the yield in each isolate was approximately 0,25 -0,5 µg of DNA. This amount of DNA was sufficient for restriction analysis only after labelling with ^{32}P.

PROTEIN ANALYSIS: proteins from purified polyhedra were analysed by SDS/PAGE, after suspension appropriate amount of polyhedra in standard loading buffer and 3 min. boiling according to Laemmli, 1970.

RESULTS and DISCUSSION

The inclusion bodies of *Stilpnotia salicis* virus isolated from five voivodeship had no big differences in shape and size. There are dodecahedral,tristetrahedral and some are cuboidal in shape.

Analysis of SsMNPV total proteins in nine isolates by 15% SDS-PAGE revealed some differences in polypeptide pattern. Seven from nine isolates had a similar protein distribution with the major broad band of 29-30 kD. The differences could be observed in two isolates, where major protein had a molecular weight smaller than 29 kD.

Restriction products of DNAs after digestion with XhoI , labelled with ^{32}P showed several changes in the distribution during electrophoresis in 0,8% agarose. Restriction products in the range of 22,000 - 0,6 kbase pair indicate similar pattern for isolates E and Aa. In isolate Ab, eight additional fragments can be seen. The analysis of Hind III restriction products revealed also negligible differences between isolates E and D. In isolate Ab seven additional bands in the range of 4,45 - 1,05 kbase pair appeared and seven others were missed.

LITERATURE

Cherepenko, E.I., Martynenko, E.I., (1985) A simple method for baculovirus DNA isolation. Molecular biology, Moscow 19, 1519-1524

Evlachova,A.A., Svecova, O.J.,(1964) Metody rozpoznavania boleznoj nasiekomyvh. Ministerstwo Selskogo Chozajstva SSSR ss.32-33.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature 227, 680-685

Lameris, A.M.C., Ziemnicka, J., Peters, D., Grijpma, P., Vlak, J.M., (1985) Potential of baculoviruses for control of the satin moth, *Leucoma salicis L.* Med.Fac.Landbouww.Rijksuniv.Gent. 50/2a, 431-439

Ziemnicka J. (1981) Studies on nuclear and cytoplasmic polyhedrosis viruses on the satin moth *(Stilpnotia salicis L.)(Lepidoptera, Lymantriidae)* Prace Naukowe Instytutu Ochrony Roślin XXIII (1), 75-142

COMPARATIVE HISTOPATHOLOGY OF *AGROTIS SEGETUM* NPV AND GV IN *A. SEGETUM* LARVAE

R. OBALLE, E. VARGAS-OSUNA, H.K. ALDEBIS and C. SANTIAGO-ALVAREZ

Cátedra de Entomología Agrícola y Forestal
Departamento de Ciencias y Recursos Agrícolas y Forestales
E.T.S.I.A.M. Universidad de Córdoba. Apartado 3048.
14080 CORDOBA, ESPAÑA.

Summary

A histopathological study of two baculoviruses, a nuclear polyhedrosis virus (AsNPV) and a granulosis virus (AsGV), was conducted with third-instar *Agrotis segetum* Schiff. larvae which were fed with purified suspensions of inclusion bodies.

Sections of the diseased larvae showed early signs of infection 24 hours after treatment. The AsNPV inclusion bodies were detected in the nuclei of the fat body, tracheal matrix and hypodermis, and the AsGV infection was observed in the same tissues as well as to a lesser extent in the cells of the Malpighian tubules.

The tracheal matrix and hypodermis were heavily infected by both baculoviruses, whereas in the fat body only the AsGV caused a generalized infection.

1. Introduction

Two baculoviruses isolated from *Agrotis segetum*, a granulosis virus (AsGV) and a nuclear polyhedrosis virus (AsNPV), with high insecticidal activity determined by laboratory biossays, showed field efficacy for the control of *A. segetum* in maize, when applied as a bait (Caballero *et al.*, 1988; 1991; Bourner *et al.*, 1992).

The yield of the *in vivo* production of baculoviruses, in a host, depends upon the virus involved. In fourth instar *A. segetum* larvae, the AsGV showed higher productivity ratios than the AsNPV (Vargas-Osuna *et al.*, 1992; 1995; Oballe *et al.*, submitted).

The aim of this study was to compare the histopathology of these two virus diseases in *A. segetum* larvae. This research might provide more information about the potential of the *in vivo* production for each baculovirus.

2. Materials and Methods

The *A. segetum* larvae were taken from a stock colony maintained at insectary conditions (26±2°C, 60±5% RH and 16 h day length) on an artificial diet (Santiago-Alvarez and Vargas-Osuna, 1986).

The AsGV inoculum, a suspension of $2.8x10^{10}$ IB/ml, was a strain isolated from field collected *A. segetum* larvae in Montilla, Córdoba, Spain; the AsNPV inoculum, a suspension containing $2.4x10^{8}$ IB/ml, originated from Rothamsted Experimental Station, Harpenden, Herts, UK.

A. segetum larvae were inoculated according to the method of Vargas-Osuna and Santiago-Alvarez (1988). Newly moulted third instar larvae were placed singly in plastic cups (30 mm diameter) and fed on alfalfa leaf discs (5 mm diameter) that had been treated by means of a microapplicator with a suspension of IBs corresponding to the respective LDs80 for each baculovirus (Oballe, 1993). The virus suspensions were made by dilutions of the inocula in distilled water to which 0.1% Agral (a wetting agent) had been added. As a control, larvae were fed on leaf discs treated with sterile distilled water plus 0.1% Agral. Larvae that did not completely eat the treated discs during 24 hours were discarded; those that did were transferred to new plastic cups (30mm diameter) and supplied with the artificial diet without formalin. The larvae were maintained at insectary conditions.

Daily, larvae were fixed in alcoholic Bouin's fluid for 48 hours, dehydrated in a graded ethanol series, and embedded in paraffin. Each larva was transversely serially sectioned at 7 μm and stained by the method of Hamm (1966).

All positive diagnoses of infections of the tissues were based on observations of the typical inclusion bodies in the cells of the subject tissues.

3. Results and Discussion

Larvae which had been infected with AsGV became more or less swollen, and the ventral portion of the abdomen turned whitish in color in an advanced stage of infection. Soon after death, the tissues were almost completely liquified.

Nuclear hypertrophy was first observed in the fat body 24 hours after AsGV ingestion, and after 48 hours in the hypodermis and Malpighian tubules. From the 4th day post-inoculation, the infection was apparent in the fat body, hypodermis, tracheal matrix and the cells of the Malpighian tubules. Viral infection in the Malpighian tubules only was occasionally observed. The polyorganotropic nature of this disease has been previously reported by Lipa and Ziemnicka (1971) with a strain of AsGV isolated in Poland.

A. segetum larvae infected with AsNPV did not grow as rapidly as healthy larvae. A few days after treatment they became sluggish and pallid. The integument became very fragile and often ruptured before death.

Similarly to the AsGV, 24 hours after larvae had been fed with the AsNPV, fat body cells showed early signs of infection, although to a lesser extent that the AsGV infected larvae. Two days after treatment, nuclear hypertrophy was also observed in the fat body and tracheal matrix. From the 3th day post-inoculation, inclusion bodies were produced in the fat body, hypodermis and tracheal matrix. Typically, the nuclei of the infected cells were filled with polyhedra. The hypodermis and tracheal matrix showed substantial polyhedra development, whereas fat body cells appeared to be less affected. By the 8th day post-inoculation, development of the IBs was extensive and many nuclei of the infected cells had ruptured and released masses of polyhedra into the haemocoel.

Pathological changes and IB production were not apparent in other tissues examined with the light microscope, but the possibility exists that some of them may also be infected, especially at a late stage of infection.

These histopathological patterns of each baculovirus disease could explain the differences between the IB production yields obtained from *A. segetum* larvae treated with AsGV and AsNPV (Oballe *et al.*, submitted).

Acknowledgements

This research was supported by the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) grant AGR90-0445.

4. References

BOURNER, T.C., VARGAS-OSUNA, E., WILLIAMS, T., SANTIAGO-ALVAREZ, C. & CORY, J.S., 1992. A comparison of the efficacy of a nuclear polyhedrosis and granulosis viruses in spray and bait formulations for the control of *A. segetum* (Lepidoptera: Noctuidae) in maize. Biocontrol Science and Technology 2: 315-326.

CABALLERO, P., FLEISHACKER, C., VARGAS OSUNA, E. & SANTIAGO-ALVAREZ, C., 1988. Un baculovirus autóctono (virus de la granulosis; VG) de *Agrotis segetum* (Lepidoptera: Noctuidae) y sus posibilidades de empleo en la lucha contra "gusanos grises". Bol. San. Veg. Plagas **14**: 171-174.

CABALLERO, P., VARGAS-OSUNA, E. & SANTIAGO-ALVAREZ, C., 1991. Efficacy of a Spanish strain of *Agrotis segetum* granulosis virus (Baculoviridae) against *Agrotis segetum* Schiff. (Lep., Noctuidae) on corn. J. Appl. Ent. **112**: 59-64.

HAMM, J.J., 1966. A modified azan staining technique for inclusion body viruses. J. Invertebr. Pathol. **8**: 125-126.

LIPA, J.J. & ZIEMNICKA, J., 1971. Studies on the granulosis virus of cutworms *Agrotis* spp. (Lepidoptera, Noctuidae). Acta Microbiol. Polonica, Ser. B, 3: 155-162.

OBALLE, R., 1993. Comparación de la eficiencia del sistema *Agrotis segetum*-baculovirus en la producción *in vivo* del virus de la granulosis y del virus de la poliedrosis nuclear. Trabajo de Investigación Fin de Carrera. Universidad de Córdoba. 111 pp.

OBALLE, R., VARGAS-OSUNA, E., ALDEBIS, H.K. & SANTIAGO-ALVAREZ, C. Comparative production of *Agrotis segetum* GV and NPV in *A. segetum* larvae. J. Econ. Entomol. (submitted).

SANTIAGO-ALVAREZ, C. & VARGAS-OSUNA, E., 1986. Differential mortality between male and female *Spodoptera littoralis* larvae infected with a baculoviruses. J. Invertebr. Pathol. **47**: 374-376.

VARGAS-OSUNA, E. & SANTIAGO-ALVAREZ, C., 1988. Differential response of male and female *Spodoptera littoralis* (Boisduval) (Lep., Noctuidae) individuals to a nuclear polyhedrosis virus. J. Appl. Ent. **105**: 374-378.

VARGAS-OSUNA, E., CABALLERO, P. & SANTIAGO-ALVAREZ, C., 1992. The production of *Agrotis segetum* nuclear polyhedrosis and granulosis viruses in the fourth-instar *A. segetum* larvae. Proc. XXV Annual Meeting, Society for Invertebrate Pathology. Heidelberg, August 16-21. Germany.

VARGAS-OSUNA, E., CARRANZA, P., ALDEBIS, H.K. & SANTIAGO-ALVAREZ, C., 1995. Production of *Agrotis segetum* granulosis virus in different larval instars of *A. segetum* (Lep., Noctuidae). J. Appl. Ent. **119** (in press).

ULTRA HIGH PRESSURE DECONTAMINATION OF VIRAL PESTICIDES

B. KELLER[1], I. BURKHARDT[1], E. FRITSCH[1], R.G. KLEESPIES[1], P. BUTZ[2], H. LUDWIG[3], B. TAUSCHER[2], J. HUBER[1]

[1] Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany
[2] Federal Research Centre for Nutrition, Institute for Chemistry and Biology, Engesserstr. 20, D-75131, Karlsruhe, Germany
[3] University of Heidelberg, Institute of Pharmaceutical Technology, INF 346, D-69121 Heidelberg, Germany

SUMMARY

Ultra high isostatic pressure was applied to inactivate microbial contaminants of *in vivo* produced baculovirus preparations (*Cryptophlebia leucotreta* granulosis virus [*Cl*GV] and *Agrotis segetum* nuclear polyhedrosis virus [*As*NPV]). At a pressure of 600 MPa and a temperature of 50°C for 60 minutes the concentration of microbial contaminants was reduced much below the treshold value of 10^7 cfu's per gram preparation. The activity of *Cl*GV remained unchanged, however, the activity of *As*NPV was reduced by 98%. These effects were documented by electron microscopic studies.

INTRODUCTION

Ultra high isostatic pressure (UHP) is a mild sterilization technique (Tauscher, 1995) recently introduced in food industry. It kills vegetative microorganisms and inactivates enzymes without negatively influencing nutrients and the natural taste of food. First studies in application of this decontamination method in the non-food area are published by Butz et al.. We have applied this technique to inactivate microbial contaminants of insect biocontrol preparations like *in vivo* produced baculoviruses (*Cryptophlebia leucotreta* granulosis virus [*Cl*GV] and *Agrotis segetum* nuclear polyhedrosis virus [*As*NPV]) and have investigated the influence of high pressure treatment on the biological activity of the virus preparations.
Electron microscopic studies should reveal if ultra-high pressure treatment causes changes in virus structure.

MATERIALS AND METHODS

Virus Preparation
Cryptophlebia leucotreta granulosis virus [*Cl*GV] was propagated in fourth instar larvae of *Cryptophlebia leucotreta* and partly purified by differential centrifugation (Huber, 1981). *Agrotis segetum* nuclear polyhedrosis virus [*As*NPV] was propagated in fourth instar larvae of *Agrotis segetum* and purified under the same conditions as *Cl*GV.

UHP Treatment
The experiments were performed in a high pressure device consisting of 10 thermostated micro-autoclaves (10 ml, inner diameter 12 mm) connected by valves (Butz et al., 1995). Water was used as pressure transmitting medium. For the treatment samples of 1 ml were filled into tightly closed flexible polyethylene bags.
Conditions of pressure treatment were:
Pretreatment of 30 min at 80 MPa followed by 20 or 60 min, 600 MPa or 700 MPa at 50°C.

Bioassay Methods
The activities of the virus preparations were determined in 6 days bioassays with neonate larvae of *Cryptophlebia leucotreta* for *Cl*GV and 12 days bioassays with neonate larvae of *Agrotis segetum* for *As*NPV. The same diet incorporation technique was used as developed for the codling moth *Cydia pomonella* (Huber, 1981).

Microbial Counts
Surviving microorganisms were counted according to Koch's method on Standard I nutrient agar, or by using a spiral plater (spiral systems) and CM3 (Oxoid) agar plates.

Electron microscopy
*As*NPV-polyhedra treated with 700 MPa at 60°C for 60 min and untreated polyhedra as a control were prepared for electron microscopic studies. The polyhedra were fixed with glutaraldehyde and osmiumtetroxide and embedded in methacrylate. Ultrathin sections were cut with a Leica Ultracut S and studied with a Zeiss 902 electron microscope.

The pressure treatment of granulosis virus *Cl*GV was the same as for *As*NPV. Then the protein was treated with sodium hydroxide and stained with sodium phosphotungstate.

RESULTS

With the *Cl*GV preparation (Figure 1) at a pressure of 600 MPa and a temperature of 50°C for 60 min the number of germs was reduced to much below the threshold value of 10^7 cfu's per gram preparation without changing the virus activity. For the *As*NPV preparation, however, the same conditions let to an evident reduction of 98% in the titer of active virus (Figure 2). Electron microscopic studies of ultrathin sections of pressure treated *As*NPV-polyhedra showed that the virions displayed severe deformations. The virus bundles looked as if they were fused or as if they were coagulated. In addition the high pressures resulted in a strange deformation of the paracrystalline structure of the polyhedrin and often the polyhedra were disrupted. The severe reduction of the virus activity in bioassays mentioned above is evident with these results.

In contrast, the granulosis virus *Cl*GV was much more resistant against ultra-high pressure treatment. Most of the single virions in the capsules kept their normal shape. But a few deformations were found. Some virions had been dissolved or were swelled, others were pressed out of their capsule. Again, the paracrystalline structure was destroyed and some capsules were splitted and disrupted.

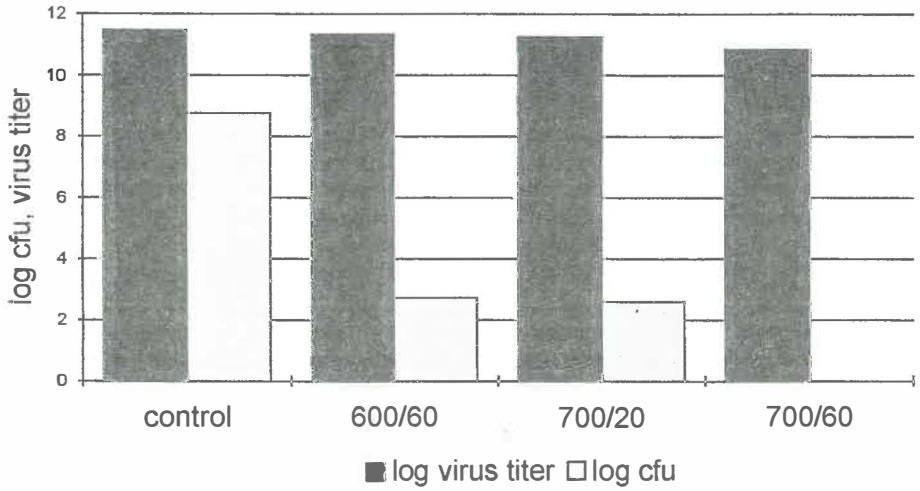


Figure 1: $\log_{10}$ virus titer of *Cf*GV preparation in comparison to $\log_{10}$ colony-forming units after pressure treatment at 50°C with pretreatment at 80 MPa for 30 min. Conditions of pressure treatment for each sample (pressure in MPa / time in min) on abscissa.

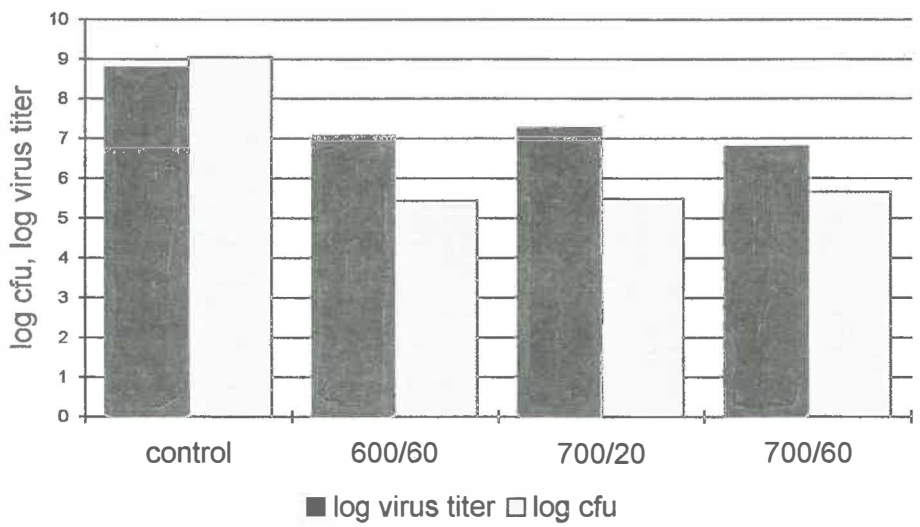


Figure 2: $\log_{10}$ virus titer of *As*NPV preparation in comparison to $\log_{10}$ colony-forming units after pressure treatment at 50°C with pretreatment at 80 MPa for 30 min. Conditions of pressure treatment for each sample (pressure in MPa / time in min) on abscissa.

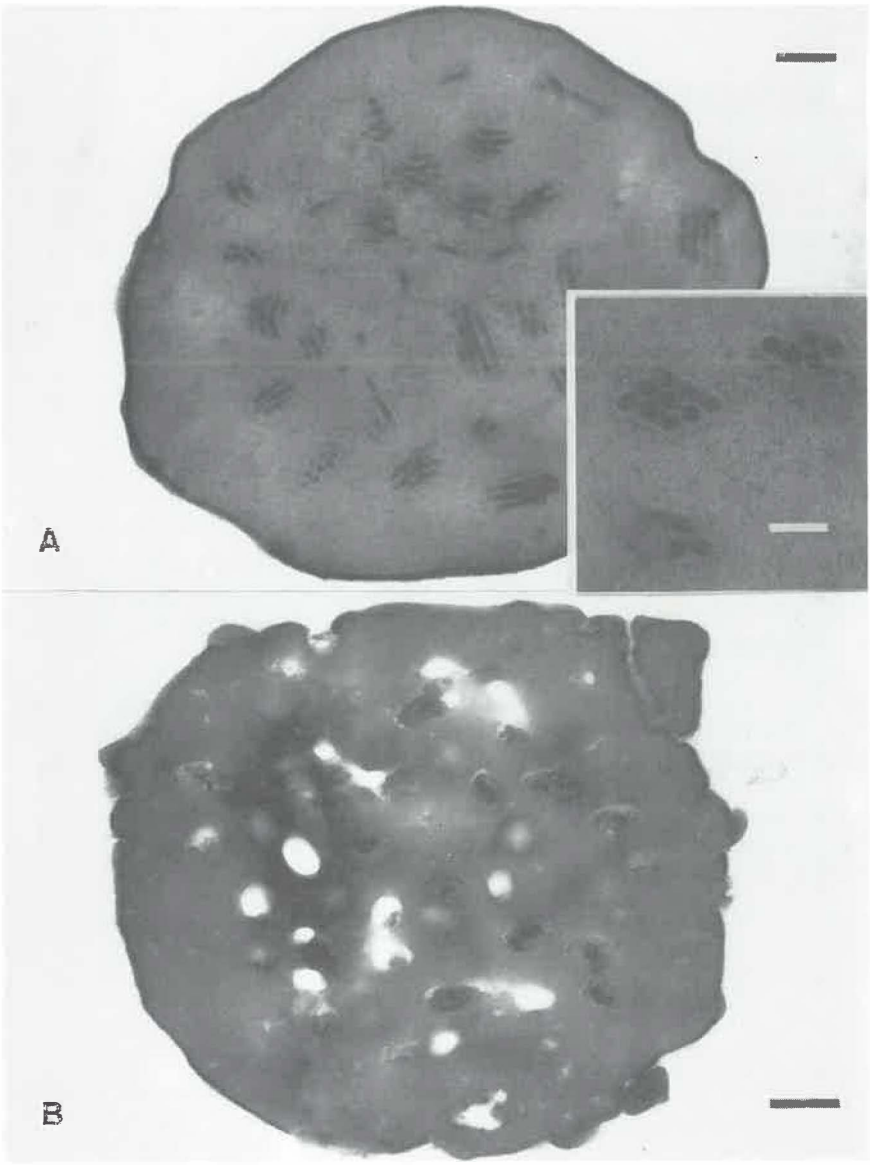


Fig.3: Ultrathin sections of (A) a non-treated *As* NPV-polyhedra. The paracrystalline structure of the polyhedrin is visible and the occluded virus bundles are well developed. (Bar = 0.2µm.) Insert: Part of an untreated polyhedron displaying virus bundles embedded in paracrystalline polyhedrin. (Bar = 0.1µm.) (B) *As*NPV-polyhedra treated with 700 MPa at 60°C for 60 min. The paracrystalline structure of the polyhedrin is destroyed. The virus bundles are totally collapsed. (Bar = 0.2µm.)

CONCLUSIONS

Whereas the microbial contamination of virus-preparations containing granulosis viruses can be reduced by ultra high pressure treatment without loss of virus-activity, nuclear polyhedrosis viruses seem to be more sensitive to UHP and loose most of their activity. This novel method for decontamination of viral pesticides can, therefore, only be recommended for preparations based on granulosis viruses.

REFERENCES

Butz, P. , Fritsch, E. , Huber, J. , Keller, B. , Ludwig, H. , Tauscher, B. , 1995. Ultra high pressure decontamination of insect biocontrol preparations. Biocontrol Science and Technology 5 : 243 - 246.

Huber, J. , 1981. Apfelwickler-Granulosevirus : Produktion und Biotest. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie 2 : 141-145.

Tauscher, B. , 1995. Pasteurization of food by hydrostatic high pressure : chemical aspects. Zeitschrift für Lebensmittel - Untersuchung und - Forschung 200 : 3-13.

6. Entomopathogenic Bacteria

Spectrum of action and efficiency of BT

O.V. Smimov

ALL-Russian Research Institute for Agricultural Microbiology, Podbelskogo, 3, 189620, St.-Petersburg-Pushkin, Russia

Summary

Bacillus thuringiensis is a polytypic species that has a great potential for control of dangerous pests that have never been targeted in microbial control before. The study of new strains and pathotypes of BT allows us to create new biocontrol products with great perspectives. For example: Bactoculicide - against harmful Diptera and Bacicol - for control of Coleopteran pests. In order to solve the problem a wider spectrum of activity biopesticides it is necessary to complete a broad program of isolating and testing of BT strains among the members of regional sections of IOBC.

Introduction

The development of production and application of microbial means of pest control runs into two main problems. The first one is the question of the quantity of microbiological product, the problem of sufficient supply and production for testing.

The second important problem is the question of control of the most harmful pests by the means of biopesticides. Here, the role of science is extremely important because it's necessary to isolate and study either principally new microorganisms or to find new fields for use of the known ones and then to transfer obtained scientific information to the first category of problems. The problem with creating new preparations is that it is highly labour-intensive, but the main advantage of microbial pesticides is their selectiveness. We cannot depend on searching for wide action in microorgansims, otherwise a new round of products will take the place of the present chemicals with the only difference that they are produced with biotechnology in stead of chemical synthesis.

The solution to biopesticides widening their areas of use is primarily the question of obtaining strais with a definite spectrum of action. Their sources may be expedition gaining of dead insects or various substrates, collections of microorganisms, or any material taken from nature. There are many reasons to consider, that in such various groups of microorganisms there will be strains possessing efficiency not only for Lepidoptera, as it was before, but also for all harmful groups of pests which are a real practical problem.

Materials and Methods

The screening procedure includes the testing of collections and different samples from nature as well as aspects of their capacity to produce biopesticides. The insecticide activity, physiological and technological characteristics of strains were studied.

Results and Discussion

Bacillus thuringiensis is just the example to prove this idea. The first place as far as application practice is concerned belongs to this microorganism. But for a long time BT preparations have been characterized with more or less the same spectrum of action, which would be widened when endotoxinogenous strains are used.

All Bt strains and subspecies, that are the basis of such preparations as Bactospeine, Dipel, Dendobacilline, Lepidocide, Gomelline, Entobacterine and Bitoxibacilline are united into pathotype A - Lepidopteran pathogens. After a long period of practical application of only Bt preparations which are produced on the basis of this pathotype strains, in 1976 the isolation of subspecies *israelensis*, possessing activity agains Dipteran insects, became a real sensation. It served as an impulse for the elaboration of a new strategy of biological control of bloodsucking Diptera. A bactoculicide preparation was elaborated in Russia; it is effective not only against mosquitoes and flies, but it is applied in plant protection as well (rice, agaris mushroom mosquitoes and other species; Tab.1). Dipteran control was solved, as Bt preparations are safe for human beings. *B.t.israelensis* is harmless for fish and non-target hydrobionts. On the basis of various strains of these subspecies numerous bacterial larvicides are being produced (Bactimos, Technar, Vectobac, Moscitur, Bulmocide and others). The discovery of this microorganisms group allowed to speak about the new pathotype of Bt - pathotype B

Table 1. Efficiency of Bactoculicide against the rice gnat larvae (aeroplane treatment, Crimea).

Variants	Number of pest population per sq. met	Larvae mortality (%) on the 4-th day after treatment
Bactoculicide 2,5 kg/ha	264	90,4
Control without treatment	210	Increase of population in 1,8 time

Soon, the discovery of subspecies *tenebrionis,* possessing a specific action for beetles, allowed to base pathotype C. These microbes soon found their practical use and served as a basis for a number of preparations against harmful Coleoptera, for example, Novodor, M-1, Colorado, Decimide. In ARRIAM Bacicole is created, it possesses high efficiency against Colorado potato beetle (Tab. 2) cruciferae fleas, strawberry - raspberry weevil and other pests of the Coleoptera order. Bacicole makes it possible to protect potato and tomatoes, cabbage and other cruciferous vegetables, cereals and berry-fields.

Table 2. Efficiency of Bacicole against the Colorado beetle (larvae 1-2 instrars) on potato (North Osetya).

Variants	Decrease of population (%) on 10-th day after treatment
Bacicole - 2 kg/ha	94
Bitoxybacilline - 2 kg/ha	96
Carbofos - common practice dose	89
Control without treatment	11

Such results in the practical application of different BT-strains are well-explained. BT is a polytypical species, including more then 30 subspecies, a great number of strains with various physiological and pathogenic properties. That is why it is quite realistic to expect to detect isolates possessing new characteristics, which are useful for control of pests, belonging to groups for which biopreparations were not used before, such as sucking insects, locust and phytonematodes.

In scientific sense the strict solution to the question of the specifity of any isolate has not been realized yet. Practically there are no criteria for a priori strain selection with known physiological properties such as a strain producing toxins against a certain pest. A number of physiological and biochemical properties, H-antigen serological characteristics or other discriminative characteristics of an isolate do not at all guarantee its spectrum of action nor its virulence. On the contrary, isolating and evaluating strain practice shows that the proportion of really perspective strains among isolated cultures which were identified as BT, is not really so great.

To solve the problem of creating arious biopreparations it is necessary to carry out a broad and purposeful program of strain screening. This program has a number of real difficulties. The fact is that BT activity against insects and more over its practical use in plant protection is far from being the basis of its life strategy and obviously is not involved in natural selection. The activity of this bacterium can be defined as only by chance as for example, *B.t. israelensis* was found in the Negev dessert in 1976. Therefore, a great number of tried isolates, collections of strains and other material and a broad spectrum of insect pests under treatment should always be at the experimentor's disposal. At the same time usually an experimentator has only some insects species out of his insectary and it is impossible to widen their number greatly because of the cost and the labour intensivity of artificial breeding.

That is why it's necessary to correct the system of mutual microorganism strains testing for the season of insects appearing by sending them to colleagues. This means decent relationships with colleagues and considerable trusting, as a successful experiment may result in obtaining a perspective producer, which could become a source of dividends in its commercial producing. Legal mechanisms should be worked out in detail and this would regulate the relationship between the originator and the experimentator.

The realization of this kind of program is the direct task of the International Organization for biological control and its regional sections. Large number of investigators are working successfully within its bounds, they are keeping their mutual contacts. These contacts should be widened including remote regions. One should not exclude those isolates, which were not of practical use to their originators,

but which would have had practical value in other countries. The gain would be mutual: the improvement of the ecological situation in the world on based on biopesticide use to control pests.

Conclusions

1. BT as a polytypic species that has a great potential for a new range of biopesticides capable of controling dangerous pests which have never been controled by microbial means.
2. Here is an example: Bactoculicide based on *B.t. israelensis* is highly effective against harmful Diptera and Bacicole is usefull to control Coleopteran pests.
3. The main problem is to obtain good strains as basis for the biopesticide product. It is possible by means of cooperation among and inside the regional sections of IOBC.

References

N.V. KANDYBIN, O.V. SMIRNOV, V.P. YERMOLOVA, 1994. Bactocilicide - ecologically safe bacterial larvicide having a wide spectrum of possibilities. Proceeding of International Conference "Biotechnology St. Petersburg, Russia, September 21-23, 1994, 121-122.

O.V. SMIRNOV, V.P. YERMOLOVA, A.A. STUS, 1990. Bactoculicide as a biological means for controlling the population of bloodsucking mosquitoes. Proceedings of the All-Union Research Institute for Agricultural Microbiology "Microbiological aspects of environment protection under intensive agriculture", 60, 147-156 (In Russian).

APPLICATION HORIZONS OF CRYSTALLIFEROUS BACILLI FOR CONTROL OF PEST INSECTS, NEMATODES AND MOSQUITOS

V. SHEVTSOV, E.SCHYOLOKOVA, O.KRAINOVA, S.JIGLETSOVA AND V.ICHTCHENKO

Biotechnological Innovation Centre, 102 Lenin str., Serpukhov, 142283, Russia.

Summary

On the basis of asporogenic strain *Bacillus thuringiensis* sv.*kurstaki* ecologically safe biopreparation Asthur has been developed. Biological activity of Asthur relating to different lepidopterous pest insects of vegetables, fruits, medicinal, forest and decorative crops was on one level with sporogenic analogs. The ecological aspects of application of Asthur have been investigated. No influence of the preparation upon the entomophages has been registered. It has been established that Asthur was effective against aphids such as *Aphis gossypii, Myzodes persicae, Brevicoryne brassicae.*
Preparations on the basis of different crystalliferous bacilli have not been active in regard to free-living nematodes *Panagrellus sp.*, but they have rather high toxicity toward the parasitic phytonematodes *Aphelenchus avenae* and *Meloidogine hapla*. These preparations were very effective in regard to parasitic nematodes larvae of animals - *Nippostrongylus braziliensis* as well. The formulations without viable spores but with β-exotoxin (Thuringin) or δ-endotoxin (Asthur) were the most active.

Introduction

We have been the witnesses of the discovery of new subspecies and strains of *Bacillus thuringiensis* (Bt) and *B.sphaericus* (Bs) recently as well as on extension of application area of crystalliferous bacilli for pest control have taken place (Feitelson *et al., 1992).*
The use of bacilli against insect pests difficult to control with biopesticides are represented the most interest (Weiser, 1994).
The results of testing of crystalliferous bacilli application for control of nontraditional objects (phythonematodes, sucking pest and parasitic nematodes of animals) have been described in this study.

Materials and methods

Bt sv. *kurstaki* asporogenic strain used in these studies was selected by us.
Assays were performed with liophilized biomass of some crystalliferous bacilli Bt sv.*kurstaki*, sv.*dendrolimus*, sv.*thuringiensis* and samples of commercial preparations.
For testing we have used larvae of phytopatogenic nematodes (*Aphelenchus avenae, Meloidogyne hapla*) and larvae of animal parasitic nematodes (*Nippostrongylus braziliensis*). For bioassays in vitro 0,1 ml water with 20-30 larvae were placed in plastic little cup. Then equal volume of suspension of each preparations were added in the cup. After 72 hr of exposure at 28-30 °C mortality readings were taken with the help of binokular. In vivo bioassays in regard to *Nippostrongylus braziliensis* have been made on white mice (Fedyanina L. *et al.*, 1993).

Results and Discussion

Asporogenic bioinsecticides - next stage of ecological safety in pest control

We have obtained *Spo⁻Cry⁺* mutant Bt sv. *kurstaki* with toxicity at the initial spore parent strain level. Electron microscopy of thin section of *Spo⁻Cry⁺* mutant revealed that mutant was blocked at stage III of sporulation. No evidence of cortex synthesis was visible.
The formation of toxic components was not violated and proceeds as it was with the parent strain. *Spo⁻Cry⁺* mutant produced tipical bipiramidal-shaped crystalline inclusion bodies. The mutant development cycle ended with the cell lysis and the entry of protein crystals into the culture medium.
On the basis of the sporeless mutant the asporogenic ecologically safe biopreparation Asthur has been made in several manufacture forms: a wettable powder (WP) and liquid concentrate (LC). The comparative laboratory tests have shown that as regards biological activity relating to different test-objects Asthur was on one level with sporogenic analogs.
Laboratory small scale and field tests of Asthur effectiveness in different geographic areas of the CIS against a wide range of lepidopterous insect pests have been carried out. Its biological activity was shown at sporogenic biopreparation Lepidocide level against insect pests invading cabbage (cabbage white butterfly, cabbage moth), leaf-chewing pests of orchads and berry crops (apple moth, fruit moth, gipsy moth), forest and decorative plants (american white batterfly, green oak totrix).
Within a 3-year period Asthur has been tested with six medicinal cultures and showed the same effectiveness as a

concentrated Lepidocide, but surpassed over Dendrobacilline and Bitoxibacylline relating beet webworm, lucerne cutworm. Asthur has shown a higher level of activity than Lepidocide against winter moth and against leaf roller. The liquid concentrate has shown its high effectiveness, and with the consumption norm 1,5-3,5 times higher has been as active as wettable powder.

No influence of Asthur upon the entomophages has been registered (coccinelidae, flower flies, lacewing, ladybird and seven-spotted beetles). Out of ladybird larvae treated by the preparation full-fledged adult species have developed. The formulation is characterised by the least toxicity as compared with the sporogenic biopreparations (Lepidocide, Bitoxibacilline, Thuricide) as regards to seven-spotted ladybird larvae, widening the possibility of its application together with aphidophages.

Field trials of Asthur for control of aphids were carried out at Research Institute of Vegetable Growing (Krasnodar, Russia). Asthur-(WP) was tested against the cabbage aphid (*Brevicoryne brassicae*) and green peach aphid (*Myzodes persicae*) on seed cabbage in glass-house. Asthur-LC was tested in regard to the melon aphid (*Aphis gossypii*) on cucumber. The plants were treated by spraying. Aphids were lost within 2-3 days. Mortality was assessed after 5 days. The results of Asthur application for control of aphids are shown in Table 1.

Table 1. Efficacy of Asthur for control of aphids

Formulation	Rate	Aphid	Crop	Area of treatment	Mortality, %
Wettable powder	3 kg/ha	*Brevicoryne brassicae, Myzodes persicae*	Seed cabbage	Glass-house, 500 m^2	85
Liquid concentrate	5 l/ha	*Aphis gossypii*	Cucumber	Open field, 2000 m^2	93

Preliminary studies indicated that active substances affecting aphids were located in supernatant of culture liquid and were not connected with the δ-endotoxin.

Perspectives of bacilli use for phytonematodes control

Samples of the commercial entomocidal formulations and experimental ones on the basis of different crystalliferous strains of Bs and Bt have been tested in regard to parasitic

phytonematodes *Aphelenchus avenae* and *Meloidogyne hapla* and free-living nematodes *Panagrellus sp.* in laboratory conditions.
These preparations have not been active in regard to *Panagrellus sp.* nematode, but they have had some activity against phytonematodes differently.
Samples on the basis of some Bs and Bt sv.dendrolimus strains were found highly effective. Mortality of nematode larvae consisted 63-97 %.
It is interesting that inactivation actions on suspension of preparations such as heat treatment (100°C, 20 min), autoclaving (1 athm, 40 min), alkaline hydrolysis (pH 12 during 1 hour) practically had no influence on nemotocidal activity.

Biocontrol of animals nematods

Interesting results have been obtained during investigation of biopreparations effect on the larvae of nematodes *N.braziliensis*.
For the purpose of learning the role of β-exotoxin in nematocide action three bacterial preparation with different content β-exotoxin were tested. It have been established that Turingin, which consist of only β-exotoxin, was the most effective. It was active even at concentration 0,1-1 % .
Among the preparation without β-exotoxin the most active were formulations without viable spores, such as Asthur-3 (based on Bt sv.*kurstaki* $Spo^{-}Cry^{+}$) and Asthur-4 (based on Bt sv.*dendrolimus* $Spo^{-}Cry^{+}$). They contain more δ-endotoxin .
Comparison of larvicidal effectiveness and preparation composition enables to make a supposition about mechanism of nematocidal action. It may be supposed that nematocidal effect is connected with exo- and endotoxines.

References

FEITELSON, J.S., PAYNE, J. & KIM, L., 1992. *Bacillus thuringiensis:* insects and beyond. Biotechnology, **10**:271-275.

WEISER J., 1994. Insect pests difficult to control with biopesticides. IOBC/WPRS Bulletin, **17**:9-16.

FEDYANINA L., SHEVTSOV V. *et al.*, 1993. Efficacy of entomopathogenic *Bacilli towards Nippostrongylus Braziliensis travassons* 1914 larvae. J. Medical Parasitology and diseases, 1:16-18.

COMPARATIVE EVALUATION OF ECOTECH PRO 07,5 OF AND FORAY 02,2 UL* ACTIVITIES AGAINST *LYMANTRIA MONACHA* AND *DENDROLIMUS PINI* LARVAE UNDER LABORATORY CONDITIONS

Henryk Malinowski

Forest Research Institute, Bitwy Warszawskiej 1920 r nr 3, 00-973 Warsaw, Poland

The bioactivity of a new generation of *Bacillus thuringiensis* product, Ecotech Pro 07,5 OF (strain EG 2348, transconjugant *B.t. kurstaki x B.t. aizawai*, potency: 24 000 i.u./mg) against *Lymantria monacha* and *Dendrolimus pini* larvae in comparison to that of Foray 02,2 UL (*kurstaki*, potency: min. 11 300 i.u./mg) was evaluated under laboratory conditions. Both preparations used in identical concentrations (2,5, 5, 10 and 20 ml/l) showed a good activity against the examined larvae. Taking into consideration the speed of action it may be assumed that Ecotech Pro 07,5 OF acted faster than Foray 02,2 UL due to its higher potency.

Introduction

Outbreaks of the nun moth, *Lymantria monacha L.* the one of the most dangerous defoliating insect pests cause the severe damages to Scots pine, Norway spruce and other conifer and decidous trees in Poland and other European countries. The greatest outbreak of the nun moth, unprecedented in the history of Polish and European forestries, took place in Poland between 1978 and 1983 when chemical treatments were applied to about 3 million ha (Burzyński, Śliwa, 1984).

The periodic outbreaks of the pine moth, *Dendrolimus pini L.* sometimes cover considerable areas of Scots pine stands. In Poland this pest was controlled in an area of 6.000 and 4.000 ha in 1983 and 1993 respectively.

The main method of controlling the above mentioned pests was the application of pyrethroids which are characterized by lack of selectivity. In a continuing search for environmentally acceptable control agent to forest pest insects, the activity of a new *Bacillus thuringiensis* product, Ecotech Pro 07,5 OF, against *Lymantria monacha* and *Dendrolimus pini L.* larvae in comparison to the known product of this group, Foray 02,2 UL (=Foray 48 B), was tested under laboratory conditions.

1

* In Poland, Foray 48 B is registered as Foray 02,2 UL

Materials

The biopreparations used were:
1. Ecotech Pro 07,5 OF (strain EG 2348, transconjugant B. t. kurstaki x B. t. aizawai, potency: 24.000 i.u./mg) (Ecogen)
2. Foray 02,2 UL (B. t. kurstaki, potency: min. 11.000 i.u./mg) (Novo Nordisk)

Bioassays were carried out on the following species of insects:
1. *Lymantria monacha L.* (larvae of second/third instars)
2. *Dendrolimus pini L.* (larvae of first/second instars)

Methods

Aqueous suspensions at four concentrations (20, 10, 5 and 2,5 ml/l) were prepared for each insecticide. Bunches of Scots pine twigs about 15 cm long were dipped for five seconds in each concentration. After drying, the larvae were put on the treated bunches. The bunches with the larvae were placed in water under glass tubes covered with cloth. 30 - 45 larvae in two or three replications were used in each concentration.
The same procedure was used for controls treated with water only. Percent mortalities were calculated 2, 4, 6, and 8 days after treatment. According to the need, the results were corrected for the corresponding control values using the Abbott formula. The experiments were conducted at a temperature of 25°C.

Results and discussion

Results presented in Fig. 1 showed that Ecotech Pro 07,5 OF in comparison to Foray 02,2 UL caused higher percent mortality of *Lymantria monacha* larvae in subsequent days after treatment. Taking into consideration the speed of action, it can be stated that Ecotech Pro 07,5 OF caused 100% mortality 6 days after treatment at three higher concentrations (5, 10, 20 ml/l) while Foray 02,2 UL caused about 100% mortality 8 days after treatment at the highest concentration (20 ml/l) only. It may be concluded that Ecotech Pro 07,5 OF acted quicker against *Lymantria monacha* larvae and at lower concentrations than did Foray 02,2 UL. The tendency towards a higher activity of Ecotech Pro 07,5 OF in comparison to Foray 02,2 UL was also observed in experiments with *Dendrolimus pini* larvae (Fig. 2). A better activity of this biopreparation was more pronounced at the lowest concetration (2,5 ml/l).
A better activity and speed of action of Ecotech Pro 07,5 OF in comparison to Foray 02,2 UL may be due to its higher potency and to differences connected with strain properties.
To obtain the efficient protection of forest against *Lepidoptera* pests, the *Bacillus thuringiensis* products ought to

be first of all characterized by high potency, because the dose acquisition by the larvae is limited by feeding inhibition associated with the dose initially ingested (Frankenhuyzen, 1990).

Conclusions

- Ecotech Pro 07,5 OF showed a good activity against *Lymantria monacha* and *Dendrolimus pini* larvae.
- Ecotech Pro 07,5 OF in comparison to Foray 02,2 UL acted quicker, especially on *Lymantria monacha* larvae under laboratory conditions of that experiment. The same effect was also occured with *Dendrolimus pini* larvae but at the lowest concentration (2,5 ml/l) only. This may be due to its higher potency and strain properties.

References

- Burzyński J., Śliwa E. 1984. The threat to Polish forests by *Lymantria monacha L.* between 1978 - 1984 and the ways of combating it by Dimilin in particular. Proceedings of the Dimilin Forestry Seminar, 7 - 8 Nov. 1984. Amsterdam, Holland, 30 - 32.
- Frankenhuyzen K. Van, 1990. Can. Ent. 122: 69 - 75.

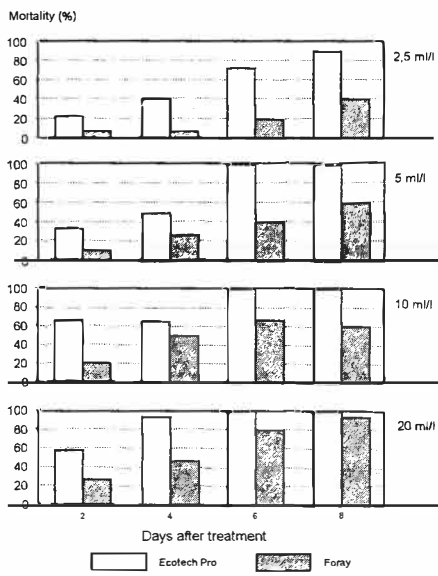


Fig. 1. Activity of EcotechPro 07,5 OF and Foray 02,2 UL against the second/third instars of Lymantria monacha larvae.

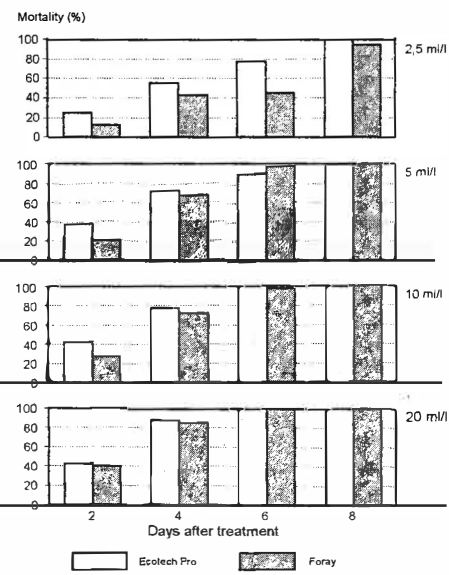


Fig. 2. Activity of EcotechPro 07,5 OF and Foray 02,2 UL against the first/second instars of Dendrolimus pini larvae.