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WORKING GROUP "INSECT PATHOGENS AND INSECT PARASITIC NEMATODES"

GROUPE DE TRAVAIL "LES ENTOMOPATHOGENES ET NEMATODES PARASITES D'INSECTES"

SECOND EUROPEAN MEETING "MICROBIAL CONTROL OF PESTS"

ROME, 6 - 8 MARCH 1989

EDITED BY
EDITE PAR *H. F. EVANS*

IOBC / WPRS BULLETIN
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INTERNATIONAL ORGANIZATION FOR BIOLOGICAL AND INTEGRATED CONTROL OF NOXIOUS ANIMALS AND PLANTS

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Introduction

This bulletin contains the proceedings of the second meeting of the IOBC/WPRS Working Group on Insect Pathogens and Insect-parasitic Nematodes. The meeting took place in Rome in March 1989.
The main themes of the meting were:

a) Current status of microbial control in practice and the registration of insect pathogens and entomoparasitic nematodes

b) The future use of genetically manipulated or non-indigenous insect pathogens and entomoparasitic nematodes

c) Microbial control of *Otiorhynchus spp*. and other soil pests

d) Environmental persistence of pathogens and nematodes

Furthermore, there were round table discussions on entomoparasitic nematodes and *Bacillus thuringiensis*.

I would like to thank Dr. Katalin Deseö and her staff for the excellent manner in which they organised the meeting with strong support from ENEA in the pleasant facilities of Domus Mariae in Rome.

The meeting was attended by a large number of scientists active in research on microbial pest control. Now that the working group is successfully established and, following recent changes in my responsibilities, I stood down as convener in September 1989. I would like to wish the new convener Dr Jürg Huber, every success.

C.C. Payne

convener

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TABLE OF CONTENTS

1. CURRENT STATUS OF MICROBIAL CONTROL IN PRACTICE AND THE REGISTRATION OF INSECT PATHOGENS AND ENTOMOPARASITIC NEMATODES

CURRENT STATUS OF MICROBIAL CONTROL IN PRACTICE AND REGISTRATION OF INSECT PATHOGENS AND ENTOMOPARASITIC NEMATODES IN BELGIUM

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Summary

In Belgium the use of microbiological means of control against insects is very limited. *Bacillus thuringiensis* Berliner based products are the only ones on the market. Three of them are serotype 3a 3b based and one is serotype H14 based. In addition, a serotype H14 based biopreparation principally used in the control of black flies has been used since 1986 to control *Simulium ornatum* populations breeding in a Belgian stream polluted by a dairy industry.

There are no specific data requirements for biological pesticides in Belgium. All insect pathogens and entomoparasitic nematodes are given the same status as chemical pesticides but parasitoids and predators are on open sale. In fact, the Belgian Committee for Registration of Pesticides waits for the directives on that subject submitted on Feb 16 1989 to the Council by the European Commission, to be put into practice.

CURRENT STATUS OF MICROBIAL CONTROL AND REGISTRATION IN DENMARK

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Production

In Denmark two companies are involved in development and production of microbiological products: NOVO and Chr. Hansens Biosystems.

NOVO concentrates at present on *Bacillus thuringiensis* only. NOVO ferments all three pathotypes and formulates the products Biobit (*B.t.kurstaki*), Skeetal (*B.t.israelensis*) and Novodor (*B.t.tenebrionis*). In 1988 only Biobit and Skeetal were sold commercially totalling 10 million Danish crowns (end-user prices) (= 1.3 mill.US$). NOVO is heading for a considerable part of the potential *B.t.k.* market focusing in particular on forest lepidopteran pests. The potential market for *B.t.i.* might be larger, but difficult to get access to. The potential market for *B.t.t.* is concentrated in southern and eastern parts of Europe and some parts of the USSR.

Chr. Hansen Biosystems has elaborated a new product based on some new strains of *Verticillium lecanii*. This product is not sold commercially yet, but is still under test. The product (a spore powder) is suspended in a basin of water and flower-seedlings are immersed into this suspension before transplanting. Using this procedure aphids and white flies should be controlled for a while. It is stressed that this procedure cannot be used in greenhouses together with pesticides; only other biological control agents such as *Encarsia formosa* and predatory mites must be used simultaneously.

In addition to the *Verticillium* product, Chr. Hansen Biosystems imports nematodes (*Steinernema bibionis*) from the USA. These are sold to control sciarid flies (*Sciaridae/Lycoriidae*) in seedlings.

Practical Use

The *B.t.* products Biobit, Dipel and Vectobac have been sold in Denmark for a few years. Biobit is sold in very small amounts (few hundred kilos) and the latter two in amounts of a few thousand kilos. Dipel and Vectobac is imported by CILLUS from Abbott Laboratories, USA.

Registration

There are no requirements for registration of microbial control agents in Denmark. Up to the present time, any such product based on the biological activity of a microorganism can be marketed. In 1984-86 a committee made a series of suggestions for registration based mainly on the ones existing in the US and UK. These suggestions have not yet become to law, but probably will be within 1.5-2 years.

CURRENT STATUS OF MICROBIAL CONTROL IN FINLAND, SWEDEN AND NORWAY

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Microbial control in practical use in the Nordic countries is still in its infancy, but apparently awakening. Only a few products are available at the moment, and a few more are in the process of being registered (Table 1). The use of *Neodiprion sertifer* NPV (NsNPV) in Finland has the longest tradition, whereas most other products have only become available during the past few years. Some products that were once registered, for example *Phlebia gigantea* against *Fomes annosus* in Finland, have already disappeared from the market. This was due to the lack of interest in the product, which itself was effective).

Research and large scale experiments are being (and have been) done on many new, potentially useful microbial agents, such as NsNPV in Sweden (S), *Lymantria monacha* NPV (S), *Agrotis segetum* GV (S), *Verticillium lecanii* (SF, S), *Beauveria bassiana* (SF, S), *Metarhizium anisopliae* (SF, S), and *Neoaplectana* spp (SF). Some of these, as well as products from other countries, are expected to enter the registration process and/or the market in the near future, probably at the rate of one to two products per year for Sweden and Finland. Note that no products are available in Norway, and that no products are expected, either, within the next few years.

There are currently no particular regulations concerning registration of microbial pesticides in any of the Nordic countries, but the products are inspected in principle according to the same protocol as chemical pesticides. These regulations are rather similar for all three countries, and the system is outlined in Fig. 1 for Finland. Sweden has designed a very thorough protocol for the registration of microbial pesticides, and this will probably be adopted by the other countries. The Nordic registration authorities will meet in summer 1989 to agree on such uniform procedures. Note that nematodes as control agents do not need to be registered as pesticides, and are free from such protocols.

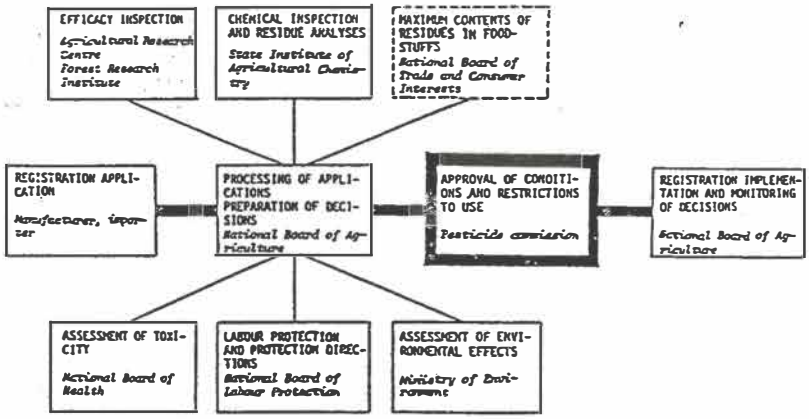

Fig. 1. Outline of pesticides approval procedure in Finland. Source: Blomqvist, H. 1986. Annales Agriculturae Fenniae 25: 37-42.

Table 1. Products presently or soon available for microbial control in Finland (SF), Sweden (S)*, and Norway (N)** (status at the beginning of 1989).

Products registered and/or in use:

Trade name	Agent	Target(s)	Available	Remarks (country,use)
Monisarmiovirus KEMIRA OY	NsNPV	*Neodiprion sertifer*	1970 (reg.1980)	SF; annual use on 4-4000 hectares
Karpasbakteeri FARMOS OY	*Bacillus thuringiensis* serotype 1	house and stable files	1981	SF; temporarily withdrawn from market for a formulation change
Nemalogic BIONEMA AB	*Neoaplectana carpocapsae*	Sciaridae in glasshouses	1987	S
	Otiorhynchus sulcatus			
Delfin SANDOZ AG	*Bacillus thuringiensis* kurstaki	Lepidoptera on ornamentals, outdoors	1988	S
Binab-T BINAB AB	*Trichoderma spp.*	*Stereum purpureum*	?	S; annual use "very limited"

Products being registered:			Remarks
Mycostop KEMIRA OY	*Streptomyces*	*Fusarium oxysporum* *Alternaria brassicola*	SF. Application filed in 1982. The approval of National Board of Health still needed to grant registration. Expected any time
Muschabac FARMOS OY	*B.t.*serotype 1	house and stable files	SF. Same as "Karpasbakteeri", but a dry powder formulation. Soon?
Delfin SANDOZ AG	*B.t.*kurstaki	Lepidoptera in vegetables, also in glasshouses	S. Extension of present permit, process completed, expected on the market in 1989.
? SANDOZ AG	*B.t.*aizawai	*Galleria mellonella*	S. Registration process almost completed; expected in 1989 or 1990.

*) Barbro Nedstam, personal communication; Berglund *et al.* 1988. Kemikalieinspektionens rapportserie Nr. 2/88, 39 pp.

**) Trond Hofsvang, personal communication.

CURRENT STATUS OF MICROBIAL CONTROL IN PRACTICE AND REGISTRATION OF INSECT PATHOGENS IN FRANCE

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The third largest agricultural chemicals market in the world is France (1.35 thousand million dollars consisting of 200 million tons of about 3000 products representing 450 active ingredients) behind both Japan (2 thousand million dollars) and the United States (4.5 thousand million dollars) and greater than the Soviet Union (850 million dollars). Nevertheless, the French market for microbial pest control products for is still restricted to the bacterial insecticide *Bacillus thuringiensis*.

In France, as well as in most major countries, the commercial development of bioinsecticides depends on the collaboration of private companies with government research agencies. Unfortunately, very few French companies have been interested in the development of entomopathogens, mainly because of their small potential markets. Only Calliope S.A. is involved in developing microbial insecticides in collaboration with INRA and ORSTOM or IRCT.

In fact, over the last few years, the situation has changed radically even if it is not obvious yet. Before considering production of genetically manipulated pathogens, scientists have begun to patent their know-how in order to ensure the profitability of product development made by private companies. As a result all microbial insecticides in a developmental phase in France are protected by patents covering aspects such as production (eg NPV), formulation (eg *Beauveria bassiana*), combinations with reduced doses of chemical insecticides in order to enhance the field efficacy of the pathogen, particularly on resistant races (eg pyrethroid-R races of Lepidoptera) and/or to extend their practical host ranges (eg *Mamestra brassicae* NPV (Table 1).

Moreover, the patent protection could cover a new use of a bioinsecticide eg *B.t.*, or the ability of a pathogen to protect a crop against a "new" target insect. The final phases of research and development include mass production, safety testing, quality control and registration. French government research needs definite financial support from private companies and the existence of patents helps to guarantee the recuperation of the investment costs for the company if the product works.

There is no specific registration procedure for pesticides in France; consequently microbial pesticides are subject to the same rules as chemical products (Fig. 1.). Nevertheless, it is essential to unify both safety testing and quality control guidelines within the EEC according to the American legislation which tends to decrease the registration requirements.

In spite of the absence of genetically manipulated entomopathogens in a development phase in France, the perspectives provided by genetic engineering should not be ignored. Thus, INRA has created its "Commission de Genie genetique". Projects of genetic manipulation have to be submitted for acceptance to this Commission. The projects can be accepted with or without modifications to their experimental design or rejected. Specific investigations are central to the evaluation of the risks linked to the use of engineered entomopathogens, mainly those devoted to their genetic stability and the consequences of any instability in the environment. In fact, the INRA authority considers any particular proposal on its individual merits without defined guidelines reflecting the lack of practical experience concerning introduction of manipulated microorganisms into the environment. Regardless, the final decision rests with the Commission of the French Ministry of Agriculture.

Table 1 : Biological insecticides in development in France

(1) Pathogen: *Mamestra brassicae* NPV
Trade name: Mamestrin R (Calliope)
Target insects and crop protection (IPM):
- lepidopterous pests of cotton *(Heliothis* spp; *Diparopsis watersi, Spodoptera littoralis*)
(patent: Mamestra NPV + pyrethroids at reduced dosages for controlling pyrethroid-R lepidopterous races); *Heliothis* control on tomatoes; *Plutella xylostella* control (patent: Mamestra NPV + pyrethroids); *Phthorimaea operculella* control on potatoes (patent).
Control of both *M. brassicae* and *Spodoptera exigua*.
Production technology: 1984-1988 (INRA)
Field efficacy: 1984-1988 (INRA, IRCT, ORSTOM)
Safety testing and quality control France and USA, 1989.
Registration France (1989 for cabbage protection); Algeria (1988), Senegal (1988).
Private company implicated in the product development: Calliope SA.

(2) Pathogen: *Spodoptera littoralis* NPV

Trade name: Spodopterin R (Calliope)
Target insects and crop protection: *Spodoptera littoralis* on cotton
Development: undetermined
Private company implicated in the product: Calliope SA

(3) Pathogen: *Lymantria dispar* NPV

Target insects: *Lymantria dispar*
Production technology 1989
Development: Canada 1990, Mediterranean area 1990
Private company implicated in the product development: Calliope SA

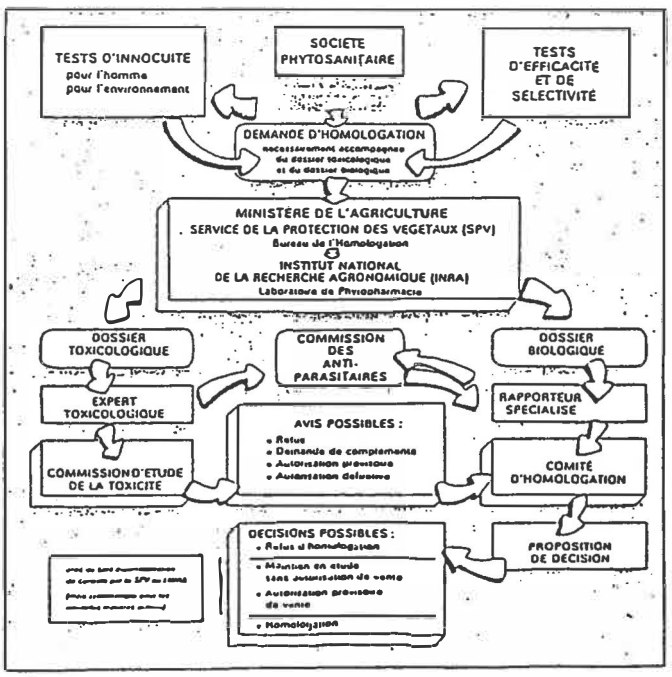
(4) Pathogen: *Cydia pomonella* GV

Product name: Carpovirusine (INRA)
Target insect and crop protection: control of codling moth in orchards
Production technology: 1983 ?
Field efficacy: 1984-1986 (INRA)
Safety testing and quality control 1988 (Calliope)
Registration: undetermined
Private company implicated in the product development: Calliope

(5) Pathogen: *Beauveria bassiana*

Target insect and crop protection: control of corn borer
Production, formulation and technology: 1984-1988 (INRA and Calliope SA)
Field efficacy: 1984-1987 (INRA, SPV, AGPM)
Safety testing and quality control: 1989
Registration: 1990 ("autorisation provisoire de vente")
Private company implicated in the product development: Calliope SA

Fig. 1 : Registration design of pesticide candidates in France
(UIPP, 1985; BOURDIN J. and COURT D, 1986)



CURRENT STATUS OF MICROBIAL CONTROL IN PRACTICE AND REGISTRATION OF INSECT PATHOGENS AND ENTOMOPARASITIC NEMATODES IN THE FEDERAL REPUBLIC OF GERMANY

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Viruses

Since 1983, Germany has official guidelines for the registration of insect viruses as pest control agents, issued by the Federal Health Authorities (BGA) and by the Federal Biological Research Centre for Agriculture and Forestry (BBA). But it is only in April 1989 that the first viral insecticide, a granulosis virus of the codling moth, *Cydia pomonella*, was granted registration status by BBA, BGA and the Federal Environmental Protection Agency (UBA). Registration is held by Hoechst, but the virus is produced by a small company in Southern Germany. The preparation will be sold under the trade-name "Granusal" and is supposed to be on the market in 1991 or 1992. There are no restrictions for its use in fruit growing, and there is no period of delay required between treatment and picking of the fruit. Though more than a dozen insect viruses have been field tested in Germany in the past, no other viruses are close to commercialisation at present.

Bacteria

There are no guidelines for the registration of Bacteria in Germany but even so, three preparations based on *Bacillus thuringiensis* have registration status and are available to the farmer: Dipel, Thuricide HP, and Neudorff's Raupenmittel (identical to Thuricide HP). *B.t.* products play only a marginal role on the pesticide market: in 1988, a total of 2500 kg was sold in Germany (100 kg for public trees and parks, 1000 kg for viticulture, 400 kg for home gardens, and 100 kg for agriculture and forestry). One of the reasons for the reluctance of the farmers to use *B.t.* preparations has to be attributed to the fact that, in Germany, these products have a restriction for use in water catchment areas (which, in the eyes of most experts, is not justified). The situation has improved a little in that, since August 1988, *B.t.* can still be used in the outer zone of water collection areas whereas, from that date, all other pesticides having any restrictions with regard to water protection zones are completely banned. The exception for *B.t.* products hopefully represents the first step towards a complete acknowledgement of the environmental safety of this pathogen in Germany.

Whereas the agricultural use of *Bacillus thuringiensis* is minimal, Germany contains the largest coherent area in the world which is being treated with *Bacillus thuringiensis israelensis* (*B.t.i*) for mosquito control. In 1988, more than 1100 ha of swamps along the Rhine river between Strasbourg and Mainz have been sprayed with several different *B.t.i.* preparations. In 11 of these, the bacterial-spores had to be killed by irradiation in order to fulfil the regulations of the health authorities.

Fungi

First contacts with health authorities have been initiated for the establishment of guidelines for the registration of mycoinsecticides but, so far, no product has obtained registration status. Most promising candidates for commercialisation are *Meterhizium anisopliae* for use against black vine weevil, *Otiorhynchus sulcatus* and *Beauveria brongniartii* against European cockchafer, *Melolontha hippocastani*. Both fungi have been extensively field testd and some safety tests have already been performed.

Nematodes

Nematodes do not have to be registered for use in insect control in Germany. Whereas their sale is not restricted, their release is regulated by the federal law on nature protection. A permit has to be obtained from the state authorities, which is unlikely to be issued for nematode species alien to the native fauna. Up to now, only the indigenous strain HL 81 of *Heterorhabditis* sp. has been sold by a German company. It proved to be very effective against larvae of the black vine weevil. Unfortunately, wider use of this nematode in the past was hindered by difficulties in mass rearing. Hopefully, this problem will be solved in 1989.

CURRENT STATUS OF MICROBIAL CONTROL IN THE UNITED KINGDOM

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Background

The United Kingdom, like most countries, relies on chemical pesticides for protection against the major insect pests. This strategy is effective, both in terms of pest control and in terms of cost. However, the environmental disadvantages of non-selectivity, dangers of resistance and problems with worker safety are increasing the pressures to find more benign methods of control. Biological control is an attractive alternative, offering a "natural" approach to pest regulation which does not harm the environment. However, despite these attributes, the use of biological control on a global scale is minute, representing only about 1% of world pesticide sales. *Bacillus thuringiensis* is the dominant biological agent with sales amounting to around 0.6% of total pesticide costs. This usage is dominated by the north American market where *B.t* is used extensively in agriculture and forestry.

Research into microbial control in the UK

Microbial control is still in its infancy in the UK and there are very few examples of large scale usage. On the other hand, research into the ecology and potential use of microbial agents has been well supported in the last 20 years or so and, despite cuts in funding for biological research, is still strong. Current research is concerned with a range of pathogens, including bacteria, fungi, viruses, nematodes and, to a lesser extent, Protozoa. Much of this research is being directed at particular environments so that, for example, the Institute of Horticultural Research are studying nematodes for control of greenhouse and mushroom house pests, viruses for control of apple pests, bacteria and fungi for control of greenhouse and other pests. The Institute of Virology is carrying out research into viral control of a number of pests of forestry, both urban and plantation, in the UK and of a range of forest and agricultural pests abroad, particularly in the tropics and sub-tropics. Other pathogens, including Protozoa, are the subject of research at various universities, often with links to IHR and IOV as the lead institutes on microbial agents.

The advances in biotechnology that have taken place during the last ten years are being reflected in the current research programmes in the above institutes. Genetic manipulation of baculoviruses and bacteria is being carried out as a research tool to increase the understanding of the ways in which pathogens act in nature. This will provide the necessary background information for full evaluation of any microbial agents whose biological activities have been modified. Thus the combined work of IOV and the Biological Control Institute at Darmstadt, West Germany is a good example of collaboration aimed at providing a risk assessment of the consequences of release of a genetically manipulated baculovirus. Although the future use of pathogens may be increasingly influenced by biotechnology, it should be remembered that the ecological consequences of introducing a pathogen,

whether manipulated or not, must be evaluated before large scale use can be contemplated.

Microbial control in practice

There has been much written about the potential of microbial agents but, unfortunately, few of these have been brought to practical fruition. This situation applies equally to the UK, despite the strong research base summarised above. The major uses of pathogens are summarised in the table below.

Pathogen	Target insects	Host plant	Commercial (C)/ Experimental (E)
B.t	Lepidoptera	Range in agri-culture/forestry	C - Dipel - Bactospeine
NPV	*Neodiprion sertifer*	Pine	C - Virox
NPV	*Panolis flammea*	Pine	E - IOV
NPV	*Euproctis chrysorrhoea*	Range of trees/ shrubs	E - IOV/Oxford university
NPV	*Agrotis segetum*	Crops, tree seedlings	E - IOV
GV	*Cydia pomonella*	Apple	E - IHR
Verticillium	Aphids, whitefly	Greenhouse plants	C - Vertalec (no longer made)
Nematodes (2 genera)	Sciarids	Mushrooms,green-house plants	E - IHR (AGC)
	Weevils	Nurseries, vines	E - IHR (AGC)
		Forestry	E - FC (AGC)
Protozoa	Range	Range	Basic research Imperial College

This table is not exhaustive but serves to emphasise that a wide range of pathogens have been used for pest control but that the number of target insects/crop types is small. The question of registration also remains unresolved with only *B.t* being fully approved for a limited range of applications. All other pathogens, with the exception of nematodes that are not currently regarded as "pesticides", have to be considered individually by the pesticide registration authorities before they can be used in the field. In addition, any microbial agent that is not native to the UK must be assessed under the Wildlife and Countryside Act that governs the release into the environment of non-indigenous organisms. In this respect any promising candidate microbial agents would have to be fully evaluated for their impacts on non-target organisms before they could be used in the field.

In conclusion, there are many promising microbial control agents available but the transition from experimental to practical use is lagging far behind the basic research. It is hoped that this position will change in the not too distant future.

THE MICROBIAL CONTROL AND THE REGISTRATION OF ENTOMOPATHOGENS IN THE IBERIAN PENINSULA

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Although as early as the last decade of the 19th century an entomopathogenic fungus, *Empusa acridii*, was applied against locusts, microbial control as a tool against pests began only in the 1950's, when entomologists became concerned with insect pathology. A limited number of entomopathogens were recorded before the 1950's in contrast with the period since when, firstly from forest insect pests and later from agricultural insect pests (approximately from the 1970's), epizootics caused by baculoviruses and some other entomopathogens were recorded; thus grew the current interest for microbial pest control.

Bacillus thuringiensis has been the most thoroughly applied entomopathogen, in experimental and commercial formulations, for both forest and agricultural pest control. Nevertheless the practical applications of *B.thuringiensis* formulations are more advanced in forest than in agricultural pest control so that research is now conducted on improvements in application techniques; for example ULV application techniques, with appropriate *B.thuringiensis* formulations, against *Thaumetopoea pityocampa*, one of the most important forest pests.

Practical experience with the other entomopathogens, baculoviruses, fungi, protozoa and nematodes, is very limited. Only some baculoviruses, in experimental formulations, are in use for agricultural pest control; a foreign strain of the *Cydia pomonella* GV has been used, in a collaborative international programme for codling moth control on apples, and four other baculovirus strains, two of the NPV type from *Spodoptera exigua* and *S.littoralis* respectively, two others of GV type from *Agrotis segetum* and *Mamestra oleracea* respectively.

Microbial control of mosquitoes and other insects of medical and veterinary importance is nearly undeveloped.

The reason for the slow development of microbial pest control in the Iberian Peninsula could be related to the low number of specialists concerned with Insect Pathology and the limited support received to promote this kind of pest control.

The microbial insecticides actually registered and available are those based on *B.thuringiensis* varieties. Nevertheless, about 1978 the viral insecticide ELCAR, based on *Heliothis zea* NPV, was registered but is no longer for sale. It is hoped that in the next few years as, in these countries, basic and applied research on insect pathology advances and more people rely on microbial control, more entomopathogens could be registered.

CURRENT STATUS OF MICROBIAL CONTROL IN ITALY: PRACTICE AND REGISTRATION

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Microbial Control

There are presently two microbial control agents on the market: *Bacillus thuringiensis* and entomogenous Rhabditids.

B.t. ssp. kurstaki is applied mostly against forest pests (*Lymantria spp., Thaumatopoea spp., Euproctis chrysorrhoea, Malacosoma neustria*) or other kind of tree-pests (*Stilpnotia salicis, Hyphantria cunea*, etc), in the vineyard against *Ostrinia nubilalis, Pieris* spp. and *Plusia* spp., etc. Furthermore, a lot of experiments are being conducted against other possible target insects. Trials are being carried with *B.t. ssp. tenebrionis* against *Leptinotarsa decemlineata* and *Epithrix hirtipennis*. The application of *B.t. ssp. israelensis* on water surfaces is also used, but at an experimental level only.

The use of entomogenous Rhabditids is promoted in glasshouses against *Otiorhynchus sulcatus* and *O.salicicola* using mostly *Heterorhabditis spp.* The recommended dosage is higher than in other countries. *Steinernema feltiae* (syn. *Neoaplectana carpocapsae*) strains are applied against the larvae of *Opogona sacchari, Epichoristodes acerbella* and other leafroller species on ornamentals and flowers.

In apple orchards against the larvae of *Synanthedon myopaeformis* the SI-100 strain of *Steinernema feltiae* is recommended for application in April, with a second application after a three-week period. Experimental formulations of this nematode are used against the treeborer larvae of *Zeuzera pyrina* in apple and pear orchards. Whilst the rate of parasitism is variable when spraying the nematodes on the whole tree, the mortality becomes high when treating individual gallery-entrances by spraying or introducing nematodes in other ways. Spraying is promoted by the Emilia-Romagna Region Authorities, but in the Campania Region there is a preference for cotton-buds soaked with biological agents, or hand-sprayers.

Trials are also being carried out against pests of different crops: eg against wireworms, Noctuids, white grubs, etc. The mixture of *B.t.* and nematodes is also under investigation.

Production

There are two companies dedicated to the production of microbial insecticides: CRC for *B.t.* and BIOERRE for entomogenous nematodes. Whilst the production of *B.t.* depends only on demand; the quantity of nematodes at present is 100 billion (10^{11}) per year, with the possibility of an increase to three times this level.

Registration

Entomogenous nematodes, being higher organisms, are exempt from registration. Due to environmental considerations, only strains collected in Europe, preferably in Italy, are produced. Importation of beneficial nematodes is at present not prohibited, but a special permit is needed.

Steinernema spp. are on the market under the commercial names: Pianbiot and Bionem S, while *Heterorhabditis spp.* are *named* Tebiot and Bionem H.

As regards *B.thuringiensis ssp. kurstaki and ssp. israelensis*, both preparations were registered for use between 1984 and 1987 under different commercial names (Bactucide, Bactospeine, Biobit*, Dipel, Thuricide and, Bactis, Bactimos, Novo Skeetal*, Tecnar, Vectobac respectively). *B.t. ssp. kurstaki* can be applied until three days before harvest.

Aerial application of *B.t.* preparations can only be done at an experimental level with the assistance of the Plant Protection Service, or by Forestry Offices, by Experimental Institutes and Universities. The same is true for field experimentation of non authorised microbial agents. Field-trials with new pathogens is strictly supervised by the different Ministry-committees.

The registration of all pesticides is the responsibility of the Ministry of Health, which collaborates with the Ministry of Agriculture and Forestry and with the Ministry of Environment. Their, so called, Plenary Committee operates through an agricultural and a medical committee. The quantities of data necessary for an application depend on the future use of the pesticide: whether it will be applied on edible or non edible crops including household. Two to three years are needed before a new active ingredient, or a new formulation, or only an extension of a registered product to a new crop, etc. receives authorisation for use on edible crops.

In order to shorten the time for the registration procedure of biological preparations, a special committee was created two years ago, reporting directly to the Plenary Committee. Nowadays, the companies interested in the commercialization of a biological product, have to prepare special documentation.

* Not yet authorised.

THE CURRENT STATUS OF MICROBIAL CONTROL IN SWITZERLAND

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Since the study of insect viruses is a research topic conducted by me and many of my students, I note with great satisfaction that the Swiss authorities were the first in Europe to register an insect virus for agricultural use.

Registration of substances and products as well as of living organisms for use in plant protection in Switzerland depends on registration by the Federal Research Station Waedenswil (FRSW). Registration or refusal results from a positive or negative evaluation by the FRSW and the Federal Offices involved in the evaluation (other Agricultural Research Stations, Federal Office of Health, Federal Office of Agriculture, Federal Office of Environmental Protection, Federal Veterinary Office). The competent board of the FRSW checks the completeness of the applicant's file of the product to be registered and decides which other Federal Offices have to be consulted. It also evaluates the preparation with regard to its suitability, efficacy and environmental compatibility. If the first evaluation is positive, the dossier is passed on to the Federal Office of Health. For unmodified microorganisms the Medical Department as well as the Department of Food Control are involved, whereas preparations based on recombinant organisms and mutants are evaluated by the Swiss Commission for Biological Safety and the Federal Office of Agriculture. At present no recombinant organisms are registered for microbial control in Switzerland. As mentioned by a member, the Commission for Biological Safety tends to refuse admittance of foreign viruses and microorganisms if they do not already belong to the microflora of Switzerland.

The registration dossier is based on the questionnaire "Evaluation and Admission of Plant Protectants with Organisms as Active Ingredients" by the Federal Office of Health, edited in collaboration with the Federal Research Stations of Waedenswil and Zurich-Reckenholz. The questionnaire contains the following 10 topics: 1. Applicant, 2. Preparation, organisms and active ingredients, 3. Specific information, 4. Chemical characteristics of biologically produced active ingredients, 5. Environmental compatibility, 6. Effects on human beings, 7. Protective measures, 8. Evidence on residues in/on food, 9. Evaluation/Registration by FAO/WHO or other countries, 10. Experiments. Except for topic 4, all topics are relevant to microbiological control agents. One to more than a dozen detailed questions are asked on each subject.

The 1987/88 edition of the annual "Swiss Index of Plant Protection Products and Auxiliary Substances Registered for Agricultural Use" contains for the first time a separate chapter on "Biological and Biotechnological Methods" with lists of the registered living organisms, pheromones, and insect traps. Beside the mite *Phytoseiulus persimilis*, the hymenopterous parasitoids *Dacusa sibirica*, *Diglyphus isea*, *Encarsia formosa*, and *Trichogramma maidis*, the list of living organisms up to 1988 names the insectivorous nematode *Heterorhabditis sp.* (registered in 1986 for use against the vine weevil) and three microorganisms.

Bacillus thuringiensis was registered in 1980 for use against winter moths and ermine moths in orchards, the second generation of the grape moths in vineyards, and the cabbage white butterfly on cabbage. The fungi

Arthrobothris irregularis and *Verticillium lecanii* were registered in 1982 against aphids in glass houses. The most recent additions to this list are a blastospore preparation of the fungus *Beauveria brongniartii*, provisionally registered in January 1988 for large scale experiments against the cockchafer, and two *Baculovirus* type 2 preparations. The first GV preparation (CpGV) with the trade name MADEX was provisionally registered in December 1988 for the treatment of 100 ha of orchards against the codling moth, *Cydia pomonella* (L.) and is now admitted for unlimited use. Another GV preparation (AoGVS) with the trade name CAPEX has been provisionally registered in February 1989 for the treatment of 50 ha of orchards against the summer fruit tortrix, *Adoxophyes orana* (F.v.R.). So far both preparations have given satisfactory control. Hopefully, the promising results will soon lead to the definitive registration of the two viruses. As stated by a member of the Swiss Commission for Biological Safety, the chances for CAPEX are especially good, AoGVS being a Swiss isolate.

CURRENT STATUS OF MICROBIAL CONTROL OF INSECTS IN THE NETHERLANDS

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Introduction

The total acreage of land in agricultural use in the Netherlands adds up to ca. 2 million hectares (Table 1). More than half of the land is in use as grassland. Other important field crops are potato, sugarbeet and corn. In greenhouses ca. 7000 hectares are used for vegetable growing and ca. 5000 hectares for ornamentals.

Table 1. Land in agricultural use in The Netherlands (data 1987)

Grassland	1,120,000 ha
Arable crops and vegetables	830,000 ha
Orchards and fruit	22,000 ha
Bulbs	16,000 ha
Greenhouse crops	12,000 ha
Tree nurseries	7,000 ha

Approximately 20 million kg active ingredient of chemical pesticides are used on this acreage annually (Table 2). Of this total 60% are soil fumigants, in particular dichloropropene used to control nematodes. The amount of insecticide active ingredient that is used roughly averages 0.5 kg per ha.

Table 2. Pesticide use in The Netherlands (data 1985)

Pesticide	Kg active ingredient	Dose in kg a.i./ha	Hectares treated
Soil fumigants	10,800,000	ca. 200	ca. 60,000
Fungicides	4,400,000	ca. 2	ca. 2,000,000
Herbicides	4,000,000	ca. 2	ca. 2,000,000
Insecticides	630,000	ca. 0.5	ca. 1,000,000

Around 200 kg of fumigant active ingredient is applied per hectare. Therefore, the number of hectares treated is also an important factor in pesticide use in the Netherlands. The data on the amount of active ingredient applied per hectare should be treated with great care as they

are estimates and are roughly pooled data covering many different compounds.

Recent political developments have put pressure on the use of chemical pesticides and it is likely that a 50-75% reduction in their use within the next 5-10 years will be enforced upon farmers and other users. This gives great impetus to the development of alternative control systems and microbials could play a major role as replacements for chemical pesticides. For the moment, however, microbials are used only on a limited scale in pest control in the Netherlands.

Practical use of microbial control agents

The only microbial control agent registered in the Netherlands is *Bacillus thuringiensis (var. kurstaki)*. Insect parasitic nematodes are also produced and used in practice but, at present for nematodes, registration as a biological pesticide is not required.

The registration of *B.thuringiensis* allows its use against various lepidopterous pests (Table 3).

Table 3. Pests for which *B.thuringiensis* is registered

Crop	Insect pest
Apple/Pear	*Operophtera brumata* *Yponomeuta malinella* *Malacosoma neustria* and various noctuids
Cabbage	*Pieris spp.* *Plutella xylostella*
Greenhouse vegetables	*Mamestra oleracea* *Chrysodeixis calchites* and other noctuids
Forest and tree nurseries	*Leucoma salicis* *Euproctis chrysorrhoea* *Yponomeuta malinella* *Lymantria dispar* *Malacosoma neustria*

B.thuringiensis is sold by various companies but not produced in the Netherlands. The total amount sold and used amounts to ca. 10,000 kg per year. It is most frequently used on greenhouse vegetables, especially against *Mamestra oleracea* and *Chrysodeixis calchites* on tomato. In particular, the biological control system that is used by the growers on more than 50% of the tomatoes stimulates the use of *B.t.* The system involves the release of the parasitoid *Encarsia formosa* to control greenhouse whitefly and predatory mites to control spider mites and thrips. The use of chemical insecticides against caterpillars would interfere with the use of these biological control agents. Asepta sporin CT is a product imported from Japan without spores which should reduce skin irritation troubling cucumber growers that use *B.thuringiensis*. Another product, namely B-401, is used to control waxmoth larvae, *Galleria mellonella* in beehives.

Table 4. Amount of *B.t.* products used in The Netherlands (data 1986-1988).

Product name	Producer	Supplier	Annual sale
Bactospeine	Solvay	Koppert	5000-7500 kg
Dipel	Abbot	Schering Aagrunol	1000-1200 kg
Thuricide HP	Sandoz	Sandoz	ca. 1500 kg
Asepta sporin CT	?	Asepta	new product
B-401	Sandoz	Sandoz	6-10 kg
Total sale			7500-10200 kg

Nematodes are commercially produced in the Netherlands by "De Groene Vlieg" and used to control *Otiorhynchus sulcatus* on pot plants. The nematode that is used is a Dutch *Heterorhabditis* species named HL'81. The yearly production amounts to 1-2 x 10^{11} nematodes. Part of the production is exported to Germany, Switzerland and Italy and used to control the vine weevil in ornamentals. A few growers use the nematodes against fungus gnats (*Sciara spp.*) and claim to have successful control.

Microbial control agents under development

An official procedure has been started for registration of *Verticillium lecanii* as a microbial control agent of the greenhouse whitefly, *Trialeurodes vaporariorum*, on greenhouse vegetables. The product will be produced and marketed by Koppert Company which has bought the rights of the former "Mycotal" product from NOVO in Denmark.

Registration by Duphar of a product based on *B.thuringiensis var. israelensis* for control of dipteran pests can also be expected in the near future. The nuclear polyhedrosis virus of *Spodoptera exigua* is the prime candidate to test the registration procedure for insect viruses, but a commercial company for production of this virus has not been found yet.

Registration of microbial pesticides in the Netherlands

Until recently microbial pesticides resided under the same registration procedure as chemical pesticides. A new procedure adapted to microbials has been developed and formulated in the "Questionnaire for approval of a biological agent used as pesticide". This questionnaire is based on the English registration criteria for biological agents.

Products based on micro-organisms (bacteria, fungi) and viruses are considered to be biological pesticides. Insect parasitic nematodes and their bacterial symbionts, predators and parasites are exempt from registration. Pheromones and toxins are considered to be chemical pesticides. Genetically modified organisms fall under separate legislation.

Four different ministries are responsible for the approval of a (microbial) pesticide. The Ministry of Agriculture & Fisheries tests the efficacy of the product, the Ministries of Health and of Environment evaluate the safety risks for consumers and the environment. Finally the Ministry of Social Affairs and Employment looks at the safety risks for the operator. All four ministries participate in the "Committee for Registration of Pesticides". The data required for registration of microbial pesticides are rather similar to the data required for chemical pesticides. The main differences from the registration procedure for chemical pesticides are:

- Efficacy criteria are less stringent.
- Group-registration for related organisms may be possible.
- Depending on the nature of the product to be registered a number of the safety tests and data may not be required.

In particular, the remark that the criteria for registration depend on the nature of the product to be registered creates uncertainties. It means that the registration procedure has to be tested by various products in order to find out which safety tests and data are necessary for registration. This means that it is impossible for a commercial company to estimate beforehand what a registration may cost or whether a certain microbial product will meet the registration criteria. On the other hand, the criteria may be handled with flexibility by the committee and this could make the registration for certain groups of microbials rather easy. Only actual applications for registration of microbials will teach us how the new procedures will be interpreted by the committee.

Sources of Information

Data on the areas in agriculture come from the Central Bureau for Statistics in The Hague. The data on pesticide use were obtained from the Ministry of Environment. The data on *B.thuringiensis* sales were obtained from various suppliers.

MICROBIAL PESTICIDES AND THEIR USE IN THE EPRS-IOBC REGION (EASTERN EUROPE)

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Introduction

From the history of microbial control it is known that first attempts at using microorganisms against economic pests were carried out by Metchnikoff, Krassilschschik, Wize and Danysz in Eastern Europe (Steinhaus 1956; Lipa 1976, 1980). I mention it to indicate that there is a long tradition of great interest in microbial control in that corner of Europe. Of course, the main reason for the present great interest in biological control of agricultural and forest pests in Eastern Europe, as in other regions, are environmental and human health problems that arise in chemical plant protection. However, it is also anticipated that in microbial, and especially in macrobial control, local resources may be used as several biotic agents can be produced or mass reared by insectary or cottage type methods. It is especially true in the cases of predatory or parasitic insects but also refers to viral, fungal and some bacterial preparations. Therefore, instead of spending hard currency to import modern chemical pesticides from abroad or to build an extremely costly sophisticated chemical industry, biological control programmes are developed and broadly introduced into practice.

The organization and operation of hundreds of bio-laboratories in the Soviet Union can serve as an illustration of that approach. At the republican, large state or cooperative farm level, these bio-laboratories produce and supply, at a very low prices, biotic agents or microbial pesticides for large scale use in plant protection. In Table 1 we can see the rapid increase in the number of bio-laboratories and the increase in areas of arable land protected by biological means (Nikonov 1986). It is estimated that, in the Soviet Union, about 5000 people are engaged in operating biocontrol. According to Nikonov (1986), biological control treatments form 30% of total plant protection actions in agriculture and forestry in the USSR. The areas protected by different biotic agents in the USSR in 1986 are given in Table 2.

The situation is similar, to some extent, in other East European countries where rather small enterprises or state laboratories, cooperatives or private owners produce biotic agents for their own use or for sale.

For this reason, I think, no other world region - except perhaps China - can compete with Eastern Europe as to the number and type of produced, registered and used microbial insecticides, fungicides, bactericides and rodenticides.

2. Production and Use of *Bacillus Thuringiensis* Products

The first *B.t.* product developed in Eastern Europe was Bathurin produced in Czechoslovakia in 1958 (Weiser 1986). However, the production was quickly stopped due to contamination problems since it was produced in an antibiotic factory.

The second product, named Entobakterin, developed in the Soviet Union about 1959, was produced on a large scale for several years. However, since *B.thuringiensis var. galleriae* is highly lysogenic the phage problems

heavily hampered its fermentation process and its production has been practically stopped. In the late sixties and early seventies, following intensive cooperation among East European specialists, facilitated by various programs, including the activities of EPRS-IOBC, has enabled the establishment of industrial production of *B.t.* products in the USSR, Bulgaria, Czechoslovakia, Poland and Roumania (Lipa 1980; Weiser 1985).

In Table 3 *B.t.* products, with their characteristics, produced in this region are listed. The technical characteristics of some products and technology of their use can be found in publications by Weiser *et al.* (1986), Weiser (1986), Anonymous (1987a,b), Kandybin and Shekhurina (1983), Kravtsov and Golyshin (1984).

The scale of production of *B.t.* products is estimated to be about 7000-8000 tons annually, the Soviet Union being the greatest producer and user. It is sufficient to say that, in the USSR, *B.t.* products are used against *Leptinotarsa decemlineata* and *Heliothis armigera* on an area of 1.8 million ha annually. Large amounts of *B.t.* are used in forest protection in the USSR against *Dendrolimus sibiricus,* in Bulgaria against *Lymantria dispar* and in Poland against *Lymantria monacha* and *Operophtera brumata*. The areas protected with *B.t.* products in some East European countries are given in Table 4.

The areas of Siberian tajga are so large that it is not technically possible to spray all trees attacked by *D.sibiricus*. Forest spraying in stripes by planes or use of *B.t.*-laden signal rockets, as spot-carriers of the pathogen, facilitates the occurrence of foci of bacterial epizootics which then spread in the tajga. This so called epizootic method of *B.t.* use seems to be the only realistic way of using microbial control against *D.sibiricus* (Kulagin 1987).

During heavy outbreaks of *L. dispar* in Bulgaria, a *B.t.* product (Dipel) was used in 1983 on an area of 59345 ha and in 1984 on 80950 ha. (Cankov and Mircev 1985).

In Poland, during the heavy outbreak of *L. monacha* from 1981 to 1985, *B.t.* products (Bactospeine, Bacillan and Thuridan) were used on areas of about 1500 ha each year, especially around lakes, due to restrictions on use of pyrethroids and other chemicals.

For the USSR, Poland and Czechoslovakia, potato growing takes up a large area of arable land, eg in Poland about 2.3 million ha and practically all plantings need to be protected against the Colorado potato beetle (*Leptinotarsa decemlineata*). Therefore, biological control of this pest, especially using *B.thuringiensis* or *Beauveria bassiana*, is very attractive or even essential (Lipa 1986; Lipa *et al.* 1989).

A *B.t.* product, Bitoxybacillin, which contains 45×10^9 spores/g + 0.6-0.8% of beta-exotoxin, provides good control of the pest but 2 to 3 treatments are necessary due to the prolonged hatching of larvae (Sikura and Sikura 1983). About 5000 tons of Bitoxybacillin are produced In the Soviet Union.

In Poland, we are in the process of determining the regions, such as around the city water reservoirs or around the national parks, where only biocontrol methods should be applied against *L.decemlineata*.

3. Production and Use of Virus Insecticides

Virus insecticides produced in Eastern Europe are listed in Table 5. As we can see practically all of them are produced in the USSR where the majority are registered (Tarasevich and Guliy 1985).

At present the scale of production of some products is not sufficient for internal use and for export. However, due to cooperative agreements, some virus products will be available for export. For example, we are, in

Poland, in the process of registration of Virin-Gjap to control the codling moth (*Carpocapsa pomonella*) (Ziemnicka *et al* 1989).

Virin LS, for control of the satin moth (*Stilpnotia salicis*), will be produced in cooperation between the USSR and Poland.

Virin-HS against *Heliothis armigera* and Virin-OS against *Agrotis segetum* are used on a relatively large scale in the USSR.

4. Production and Use of Fungal Insecticides

Although entomopathogenic fungi attract the attention of many specialists in Eastern Europe, the number of available microbial products is not large. As seen in Table 6 four products are registered in the USSR and two in Czechoslovakia. Production of Boverin in the USSR is estimated to be 5 tons per year. Production of Boverol and Verticon by three agricultural cooperatives in Czechoslovakia is estimated to be 2-3 tons per year (Weiser 1988). In one cooperative production of Boverol in 1986 was 50 kg and in 1987, 500 kg (Chlebnickova and Nesrsta 1986); the plans are to build facilities for production of 5 t/year and in future 50 t/year.

Since 1986 advanced attempts have been conducted in Poland to organize industrial production of *B. bassiana* by submerged fermentation. So far only 100-200 kg of experimental product is produced annually.

Although *B. bassiana* products can be used to control a number of pests, including the whitefly (*Trialeurodes vaporariorum*) their main use is against the Colorado potato beetle (*L. decemlineata*) (Lappa and Goral 1985). The extensive Russian literature (see Lipa 1980, 1985) and recent Czechoslovakian literature (Dirlbekova 1986) indicate that *B. bassiana* products used alone provide very variable results for *L. decemlineata* control. Therefore, official recommendations in the USSR and CSSR are to use Boverin or Boverol with reduced doses of chemical insecticides (Anonymous 1987a; Kravtsov and Golyshin 1984). A general review of biological control of *L. decemlineata* was given elsewhere (Lipa 1986a).

Verticillin, based on *Verticillium lecanii*, produced and registered in the USSR was, in 1986, used against *T. vaporariorum* on an area of 670 ha but its use is growing quickly (Nikonov 1986). Verticillin is produced in the USSR by a number of the bio-laboratories on solid media for local use so there is no problem with the so called shelf life of this insecticide.

Attempts to establish production of *V. lecanii* spores by industrial or cottage methods are being made in Bulgaria and Poland.

5. Production and Use of Nematodes

Although the East European literature on taxonomy and occurrence of entomopathogenic nematodes is large, only very few papers deal with nematode use to control pest insects. The exploratory works conducted in Czechoslovakia and Poland concern five species: *Steinernema kraussei*, *S. carpocapsae*, *Heterorhabditis cubense*, *H. bacteriophora* and *Neoaplectana bibionis*.

In Poland one private producer applied in 1989 for registration of a nematode type product named Nemix with *N. bibionis* as an active organism. This product contains also some fertilizers and is intended for home gardeners.

6. Use of Microbial Fungicides and Bactericides

In the USSR five anti-bacterial agents are registered for plant protection use: Fitolavin 100 WP, Fitobacteriomycin 2% dust, Fitobacteriomycin 5% dust, Trichotecin 1% dust, Trichotecin 10% WP. These products, depending on their formulations, are used to control some

pathogenic microorganisms infecting mainly cereals, field and greenhouse vegetables and orchard trees (Anonymous 1987a; Kravtsov and Golyshin 1984).

A microbial fungicide Trichodermin (active fungus *Trichoderma lignorum*) is used to control *Verticillium* spp. on vegetables in greenhouse and in cotton plantations.

A bacterial rodenticide Baktorodencid (a.i. *Salmonella isachenko*) is broadly used against rodents (see Table 2) (Nikonov 1986).

7. Registration Procedure Involving Microbial Products

In practically all East European countries, the registration procedure is relatively uniform and, in general, is under the control of the relevant Ministry of Agriculture. In Poland the Ministry authorized our Institute of Plant Protection to act in this area and a special unit named The Bureau of Pesticide Registration was organized for this purpose.

Parasitic and predatory insects and mites are not subjected to registration regulations since they are not considered as plant protection products, but they must fulfil quality requirements. However, the entomopathogenic microorganisms - no matter if they are produced by industrial or cottage methods - must go through a process of registration somewhat similar to a chemical product. The producer applying for registration must present, among various data, the following:

- composition of the product;
- method of growth of the microorganism and precautions to prevent contamination from other microorganisms;
- methods of assessment of activity, standardisation and quality of the product;
- toxicological evaluation of safety tests on the microorganism and on the product;
- results of field effectiveness against target pests on which effective doses and use recommendations are based.

All the pertinent data are evaluated and the State Institute of Hygiene is consulted regarding the waiting period and other safety precautions. Only after these steps have been completed is the registration permit granted by the Ministry of Agriculture, initially for 3 years and later permanently. The microbial product is then included in the official plant protection recommendation schemes.

A similar procedure exists in other countries where, usually, leading government plant protection institutes or centers are involved in the registration procedure. However, in the Soviet Union this work is done by the State Commission of Evaluation of Chemical and Biological Pesticides. This Commission has a special sub-committee for microbial pesticides which plays a highly positive role in developing microbial control products and their introduction into practice. The subcommittee receives, from individuals or institutions, proposals of newly invented products, and pilots them through production of the amount needed for field and toxicological evaluation, and sponsors the registration.

This subcommittee also seeks the best way to commercialise the product either within the microbiological industry or within the net of biolaboratories producing various biocontrol agents.

Of special importance are safety requirements with respect to microbial insecticides and entomopathogenic microorganisms. Such safety

tests and other requirements in Eastern Europe were broadly discussed by Guliy *et al.* (1986), Melnikova (1986) and Vasilieva *et al.* (1981).

References

ANONYMOUS, (1987a): Means of plant protection, defoliants, dessicants and growth regulators. Zashchita Rastenij No. 1:30-65. Microbial rodenticides pp. 51; Microbial insecticides and fungicides pp. 52-53. (In Russian).

ANONYMOUS, (1987b): Chemical and biological means of forest protection. Zaschita Rastenij No. 7:50-54. (In Russian).

CANKOV, G., MIRCEV, P. (1985): In "Biologicky a Biotechnologicky Boj se Skudci Lesa. Dum Techniky CSVTS. Ceske Budejovice, p. 27-30. (cited after Weiser 1986). (In Russian).

CHLEBNICKOVA, M., NESRSTA, M. (1986): Present status and prospects of the industrial production of Boverol. pp. 28-30. In "Myko-preparaty Ceskoslovenske Vyroby a jejich Vyuziti v Ochrane Polnich Kultur". Praha 64 pp.

DIRLBECKOVA, O. (1986): Results of testing of the effectiveness of the mycoinsecticide Boverol for the control of the Colorado potato beetle (*Leptinotarsa decemlineata* Say). pp. 36-40. In "Mykopreparaty Ceskoslovenske Vyroby a jejich Vyuziti v Ochrane Polnich Kultur". Praha 64 pp.

GULIY, V.V., LESKOVA, P.Ya., MURZA, V.I., SHTERNSHIS, M.V., IVANOV, G.M. (1986): The problems of microbiological product safety for environment and human health. No. 17:19-44. (In Russian).

KANDYBIN, N.V., SHEKHURINA, T.A. (1983): Technology of use of microbial preparations for plant protection and its development. Inform. Bull. EPS IOBC No. 6:27-40. (In Russian).

KRAVTSOV, A.A., GOLYSHIN, N.M. (1984): Preparaty dlya Zashchity Rastenii (Preparations for plant protection) Kolos, Moskwa, 175 pp.

KULAGIN, V.S. (1987): Problems of epizootic application of *Bacillus thuringiensis* in forest biocenoses. pp. 92-107. In "Entomopathogennye Bakterii i ich Rol v Zascite Rastenij". Vaschnil, Novosibirsk. (In Russian).

LAPPA, N.V., GORAL, V.M. (1985): The use of Boverin in plant protection in the USSR. Inform. Bull. EPS IOBC No. 12:47-51. (In Russian).

LIPA, J.J. (1975): An Outline of Insect Pathology. F.S.C.P.D. Warsaw, 269 pp.

LIPA, J.J (1980): Progress in Microbial Control (1955-1980). 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. (1985): History of biological control in protected culture. Eastern Europe. pp. 23-29. In "Biological Pest Control: The

Glasshouse Experience" (N.W. Hussey, N. Scopes, eds). Blandford Press, Poole, Dorset, 240 pp.

LIPA, J.J. (1986): Progress in biological control of the Colorado beetle (*Leptinotarsa decemlineata*) in Eastern Europe, Bulletin OEPP/EPPO 15:207-211.

LIPA, J.J., KOROL, I.T., SOKOLCZYK, A., BARTKOWSKI, J., DEGA, B., RATAJCZYK, G. (1989): Bitoksybacillin and Boverin in controlling the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Materialy 29 Sesji Nauk. Inst. Ochr. Roslin (in print).

MELNIKOVA, E.A. (1986): Pathogenicity of *Bacillus thuringiensis* and its formulations toward vertebrates pp. 118-130. In "Entomopatogennye Bakterii i ich Rol v Zascite Rastenij". WASCHNIL, Novosibirsk, 131 pp. (In Russian).

NIKONOV, P.V. (1986): Developing biological methods. Zashchita Rastenii No. 5:2-4. (In Russian).

SIKURA, A.J., SIKURA, L.V. (1983): Use of biopreparations. Zashchita Rastenii No. 5:38-39 (In Russian).

STEINHAUS, E.A. (1956): Microbial control, the emergence of an idea. Hilgardia 26:107-160.

TARASEVICH, L.M., GULIY, V.V. (1985): The state of affairs and perspectives for the use of entomopathogenic viruses in plant protection. Inform. Bull. EPS IOBC No. 12:64-67. (In Russian).

WEISER, J. (1986): Impact of *Bacillus thuringiensis* on applied entomology in Eastern Europe and in the Soviet Union. Mittlg. Biol. Bundesanstalt (Berlin-Dahlem) 233:37-49.

WEISER, J. (1988): Personal information.

WEISER, J., VIDENOVA, E., KANDYBIN, N.V., SMIRNOV, O.V. (1986): Technical characteristics and standardizations of microbial entomocidal preparations. Inform. Bull. EPS IOBC No. 16:44-52 (In Russian).

ZIEMNICKA, J., KANAPACKA, W., LIPA, J.J., KOROL, I.T., KOZLOWSKI, J. (1989): Infectivity of Virin-Gjap for Polish populations of codling moth (*Carpocapsa pomonella L.*) Meterialy 29 Sesji Nauk. Inst. Ochr. Roslin (in print).

Table 1. Number of laboratories producing bioagents and area protected with biological means in the USSR (1965-1985)

Year	1965	1970	1975	1980
Number of biolaboratories (with *Trichogramma* rearings)	192	372	470	723
Area protected with biomethods (min. of ha) (*Trichogramma* released)	0.4	4.3	12.4	24.7

Table 2. Area protected using biotic agents in the USSR (1986)

Biotic agents	Area (ha)
Field crops and forest:	
Bacillus thuringiensis	1,800,000
Glasshouse crops:	
Verticillin	670
Baktorodencid	970
Trichodermin	2,880
Phytoseiulus persimilis	3,600

Table 3. Microbial insecticides based on *Bacillus thuringiensis* produced in Eastern Europe (* products registered)

Product	Strain and Serotype		Exo-	Spore to x in	Country
*Bacillan (WP)	*kurstaki*	3a3b	-	$30x10^9$	Poland
*Baktokulicid (WP)	*israelensis*	14	-	?	USSR
*Bathurin 82 (WP)	*kurstaki*	3a3b	-	$25x10^9$	CSSR
*BIP (30-WP)	*alesti*	3a	-	$30x10^9$	USSR
*BIP (20-FC)	*alesti*	3a	-	$20x10^9$	USSR
*Bitoksibacillin (WP)	*thuringiensis*	1	+(0.6-0.8%)	$45x10^9$	USSR
*Dendrobacillin (20-FC)	*dendrolimus*	4a4b	-	$20x10^9$	USSR
*Dendrobacillin (60-WP)	*dendrolimus*	4a4b	-	$60x10^9$	USSR
*Dendrobacillin(100-WP)	*dendrolimus*	4a4b	-	$100x10^9$	USSR
*Dipel	*kurstaki*	3a3b	-	$30x10^9$	Bulgaria
*Entobakterin (20-FC)	*galleriae*	5a5b	-	$20x10^9$	USSR
*Entobakterin (30-WP)	*galleriae*	5a5b	-	$30x10^9$	USSR
Exotoksin	*thuringiensis*	1	++(2%)	-	USSR
*Gomelin (20-FC)	*thuringiensis*	1	-	$20x10^9$	USSR
*Gomelin (30-WP)	*thuringiensis*	1	-	$30x10^9$	USSR
*Gomelin (90-WP)	*thuringiensis*	1	-	$100x10^9$	USSR
*Insektin	*thuringiensis*	1	-	$30x10^9$	USSR
*Lepidocid (WP)	*kurstaki*	3a3b	-	$100x10^9$	USSR
Moskitur	*israelensis*	14	-	$30x10^9$	CSSR
*Thuridan (WP)	*kurstaki*	3a3b	-	$30x10^9$	Poland
*Thuridan (FC)	*kurstaki*	3a3b	-	$30x10^9$	Poland
Turindhgin	*thuringiensis*	1	-	?	Roumania
*Thuringin-1	*thuringiensis*	1	++(20%)	-	USSR
Thuringin-2	*thuringiensis*	1	++(10%)	-	USSR
Turintoks	*thuringiensis*	1	+	?	Roumania

WP - Wettable Powder; FC - Fluid-Creme

Table 4. Area protected each year using *Bacillus thuringiensis* products in some East European countries.

Country	Area in ha
USSR	1,800,000
Bulgaria	60,000
Poland	15,000[1]
Czechoslovakia	600

[1]Data for 1984

Table 5. Viral insecticides produced or tested in Eastern Europe (* products registered)

Product	Insect	Virus Type and Titer	Producer
Hifantrin	*Hyphantria cunea*	GV; $10x10^9$	Bulgaria
Mamestrin	*Mamestra brassicae*	NPV; $9x10^9$	Bulgaria
Virin-ABB	*Hyphantria cunea*	GV; $10x10^9$+NPV; $1x10^9$	USSR
*Virin-Diprion	*Neodiprion sertifer*	NPV; $1x10^9$	USSR
*Virin-EKS	*Mamestra brassicae*	NPV; $1x10^9$	USSR
*Virin-ENSH	*Lymantria dispar*	NPV; $1x10^9$	USSR
*Virin-GYAP	*Carpocapsa pomonella*	GV; $3x10^9$	USSR
*Virin-HS	*Heliothis armigera*	NPV; $7x10^9$	USSR
*Virin-KSH	*Malacosoma neustria*	NPV; $1x10^9$	USSR
Virin-LS	*Stilpnotia salicis*	NPV; $7x10^9$	USSR+Poland
*Virin-OS	*Agrotis segetum*	GV; $3x10^9$	USSR

Table 6. Fungal insecticides produced or tested in Eastern Europe (* products registered)

Product	Fungus	Titer	Country
*Aschersonin	*Aschersonia aleurodis*	$200-250 \times 10^6$	USSR
*Boverin	*Beauveria bassiana*	2×10^9	USSR
*Boverol	*Beauveria bassiana*	5×10^9	CSSR
*Boverosil	*Beauveria bassiana*	?	CSSR
Metarrhizin	*Metarrhizium anisopliae*	3×10^9	USSR
Paecilomin	*Paecilomyces farinosus*	?	USSR
*Verticillin	*Verticillium lecanii*	2×10^9	USSR
Verticon	*Verticillium lecanii*	?	CSSR
Beauveria[1]	*Beauveria bassiana*	1×10^9	Poland
Verticillium[1]	*Verticillium lecanii*	1×10^9	Poland

[1] Without trade name yet

2. THE FUTURE USE OF GENETICALLY MANIPULATED OR NON-INDIGENOUS INSECT PATHOGENS AND ENTOMOPARASITIC NEMATODES

IMPROVEMENT OF INSECT PATHOGENS BY GENETIC MANIPULATION

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Considerable potential exists to genetically modify insect pathogens and improve characteristics important for their use as control agents. Improvement could be made not only in their interactions with insects (eg infectivity/potency, lethal time, host range), but also in factors affecting production, storage, persistence and possibly in overcoming resistance when and if this is a problem.

Genetic modification can be achieved in many ways ranging from simple selection procedures, through recombination by co-replication of different strains of pathogen, to mutagenesis in either a random manner using mutagenic agents or in a site-specific manner by the insertion, deletion or modification of particular genes at defined sites on the genome.

Although some work has been done on the genetic improvement of entomopathogenic fungi and nematodes, this has so far been largely restricted to strain selection and nearly all published examples of genetic manipulation of insect pathogens concern either *Bacillus thuringiensis* (*B.t.*) or baculoviruses.

Up until now, the principal method used to generate new strains of *B.t.* in the laboratory has been a conjugation-like process during which plasmids, some of which carry toxin genes, are transferred from one strain to another (Gonzalez *et al.*, 1982). Selection from the resultant transcipients can result in strains which combine the activity spectra of both donor and recipient strains as shown in Table 1 (Burges & Jarrett, 1985).

Table 1. Bioassay data for a transcipient strain of *B.t* relative to the donor and recipient strain and the standard commercial strain, HD1.

Insect species	LC_{50} μg *B.t.*/g of diet			
	Donor	Recipient	Trancipient	HD1
Galleria mellonella	3,500	64	18.4	2,600
Heliothis armigera	48	845	44	42
Heliothis virescens	5.8	>2000	4.8	8.6
Spodoptera littoralis	>10,000	694	330	5,780
Pieris brassicae	0.98	> 100	0.72	0.64
Mamestra brassicae	>10,000	282	162	1,510

This method is limited, to some extent, by incompatibility between certain plasmids and partly because not all plasmids can be transferred in this way. These difficulties are likely to be overcome only be gene splicing techniques. A considerable amount of work is being done on the cloning and characterization of *B.t.* toxin genes and, to a lesser extent *B. sphaericus* toxin genes, and it seems likely that in the next few years these toxin genes will be engineered not only to produce new strains of *B.t.* but also to insert into an increasing range of recombinant bacteria, viruses and plants and endow them with insecticidal properties.

The host range and infectivity of baculoviruses have been altered by a variety of different methods. For example, successive passage of *Orgyia pseudotsugata* MNPV through *Trichoplusia ni*, which initially caused no mortality, eventually results in selection of a slightly modified genotype which caused high mortality of *T. ni* (Martignoni & Iwai, 1986). Wood *et al.* (1981) replicated *Autographa californica* MNPV (AcMNPV) in the presence of 2-aminopurine and then selected out a mutant strain with a lower LC_{50}, a shorter LT_{50}, and a higher yield of occlusion bodies. More attention is now directed towards site-specific mutagenesis. Most studies have involved replacement of the occlusion body protein gene with a wide variety of foreign genes simply to achieve high level expression of the protein product. This strategy is now being used to express genes which may enhance the insecticidal properties of the virus. Such genes include those coding for insect toxins or for the host insect's regulatory proteins which, when expressed at the wrong time or at relatively high level, will severely disrupt the insect's development or behaviour.

Deletion of the p10 gene in AcMNPV has also been shown to enhance infectivity (Vlak *et al.*, 1988) and as mutagenesis at this site has the advantage, for insecticidal application, of retaining the polyhedrin gene, it may prove to be of considerable value for the development of genetically-improved baculoviruses.

References

BURGES, H.D. & JARRETT, P. (1985) Preparation of strains of *Bacillus thuringiensis* having an improved activity against certain lepidopterous pests and novel strain produced thereby. UK Patent Application 2165261A.

GONZALEZ, J.M. JR., BROWN, B.J. & CARLTON, B.C. (1982) Transfer of *Bacillus thuringiensis* plasmids coding for w-endotoxin among strains of *B. thuringiensis* and *B. cereus*. Proceedings of the National Academy of Science USA 79, 6951-6955.

MARTIGNONI, M.E. & IWAI, P.J. (1986) Propagation of multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata* in larvae of *Trichoplusia ni*. Journal of Invertebrate Pathology 47, 32-41.

VLAK, J.M., KLINKENBERG, F.A., ZAAL, K.J.M., USMANY, M., KLINGE-ROODE, E.C., GEERVLIET, J.B.F., ROOSIEN, J. 7 VAN LENT, J.W.M. (1988) Functional studies on the p10 gene of *Autographa californica* nuclear polyhedrosis virus using a recombinant expressing a p10-B-Galactosidase fusion gene. Journal of General Virology 69, 765-776.

WOOD, H.A., HUGHES, P.R., JOHNSTON, L.B. & LANGRIDGE, W.H.R. (1981) Increased virulence of *Autographa californica* nuclear polyhedrosis virus by mutagenesis. Journal of Invertebrate Pathology 38, 236-241.

STRUCTURE, FUNCTION & GENETICS OF BACILLUS THURINGIENSIS ENTOMOCIDAL TOXINS

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The future development of *Bacillus thuringiensis* as a biopesticide will require improved formulations, innovative delivery systems, new strains and a comprehensive understanding of the synthesis, structure and mechanism of action of the delta-endotoxins. Armed with this understanding the techniques of molecular genetics can hopefully be used to modify the potency, specificity and durability of these proteins. Analysis of purified toxins has revealed that, in many cases, individual strains have evolved to contain several toxins of different specificity. Isolation of delta-endotoxin genes and study of cloned products in conjunction with the development of *in vitro* assay systems, now enables us to look more closely at the toxin mechanism. Our results led us to propose a common two-step mechanism of action for all the delta-endotoxins in which initial toxin binding to a cell specific receptor at the surface of the mid-gut epithelial cells is followed by insertion into the membrane to create a leakage pore 1-2 mm in diameter. Cell destruction then results from colloid osmotic lysis. Progress has recently been made in studying the nature of the receptors for different toxins and the kinetics of toxin binding, using susceptible insect cell lines and preparations of insect mid-gut membranes. Artificial membrane systems have been used to investigate the lytic capacity of the toxins and to study the molecular properties and ionic selectivity of individual toxin pores.

Comparison of the structures of cloned genes of widely differing specificity has revealed conserved sequences suggesting that the toxins may have evolved from a common ancestor. A major programme is underway to identify regions of the toxins that are responsible for specificity determination and to discover whether other regions of these proteins have a discrete role in the toxin mechanism, e.g. membrane penetration. In this task, extensive use is being made of gene manipulative techniques such as chimera construction, deletion mutagenesis and site-directed mutagenesis. Deletion mutagenesis has been especially valuable in allowing us to delineate the minimum toxic fragment within each protoxin. A number of delta-endotoxins show toxicity to two insect orders, e.g. Lepidoptera and Diptera and therefore offer us a special opportunity to locate specificity determinants using these gene manipulative techniques. Our work with several of these dual specificity toxins suggests that differential proteolytic processing by the different insect orders is the key to their broad host range. The effect of the different gut enzymes appears to be to expose one or other mutually exclusive toxin conformations capable of binding different receptors. These results also suggest that differences of only very few amino acids between two toxins can result in marked changes in specificity.

Novel *B. thuringiensis* strains will continue to arise in screening programmes, but molecular genetics can augment this search by artificially constructing new strains. This was initially achieved by exploiting the fact that conjugation between *B. thuringiensis* strains can result in transfer of plasmids containing delta-endotoxin genes. We have recently been successful in transferring genes between strains using electroporation and this promises to be a powerful method of constructing broad spectrum *B. thuringiensis* biopesticides in the future.

GENETIC MANIPULATION OF *CYDIA POMONELLA* GRANULOSIS VIRUS

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Codling moth is responsible for economic damage to apples, pears and walnuts. Previous work has shown *Cydia pomonella* granulosis virus (CpGV) to be an effective biological control agent in field trials. However, neonate larvae may penetrate the fruit during the four day infection period causing sufficient damage to reduce the quality, and storage time, of the fruit. It is hoped that use of a recombinant virus expressing the *Bacillus thuringiensis* (*B.t.*) delta endotoxin gene, will reduce feeding and kill the neonate larvae before they are able to penetrate the fruit.

To test this hypothesis, we are constructing a recombinant CpGV in which the granulin gene has been replaced by the *B.t.* toxin gene using a strategy similar to that for the AcMNPV expression vector system.

To aid virus manipulation and selection, embryonic *Cydia pomonella* (Cp) cell cultures have been established. These have been shown to be highly susceptible to CpGV infection and, after four passages *in vitro*, CpGV retains its infectivity for fifth instar larvae. A transfer vector possessing the complete granulin promoter has been constructed and the beta-galactosidase gene and *B.t.* delta endotoxin gene have been cloned into it. Attempts to produce recombinant CpGV are currently in progress using both *in vitro* and *in vivo* methods of co-transfection.

A strain of CpGV has been selected which contains only 2 ApaI sites, both of which are in the granulin coding region. Since neither the B-galactosidase nor *B.t.* toxin genes contain any ApaI sites, recombinant viruses containing these genes will possess no ApaI sites and this distinction should facilitate selection of recombinants.

TRANSPOSON MUTAGENESIS AS A POTENTIAL TOOL IN *XENORHABDUS* RESEARCH

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Xenorhabdus spp. are insect pathogenic bacteria symbiotically associated with nematodes of the genera *Steinernema* (*Neoaplectana*) and *Heterorhabditis*. They can undergo a phase shift from a primary to a secondary form, which reduces yields of the infective stage of the nematodes *in vitro*. Studies into the genetic basis of pathogenicity and phase shift in these bacteria have been restricted by lack of information on the genetics and molecular biology of *Xenorhabdus*.

Transposons are segments of DNA which are incapable of self-replication and which can insert into DNA sequences in a random manner. They contain antibiotic resistance genes which can function as genetic markers. When a transposon inserts into a gene, transcription is interrupted. This results in a mutant which has gained antibiotic resistance and lost the characteristic encoded by that gene. The location of the gene can be mapped using labelled DNA from the transposon as a genetic probe.

Transposon mutagenesis is an effective way of producing single marked mutations at random points in bacterial DNA. Transposons can be introduced into recipient cells by conjugal transfer of "suicide" vectors. These are transmissible plasmids which contain the transposon, but cannot be maintained in the recipient cell because of their restricted host range. The transposon can therefore only become established in the recipient through transfer into host DNA sequences. The aims of this study were to demonstrate that transposon mutagenesis of *Xenorhabdus* spp. is possible, and to compare the relative effectiveness of several suicide vectors.

Filter plate matings were performed between *X. nematophilus*, *X. bovienii*, *X. luminescens* and *E. coli* strains containing the plasmid vectors pLG221, pSUP2021, pGS9 or pUW964. These vectors carry the transposon Tn5. Results are presented on the relative efficiency of transfer of these vectors into *Xenorhabdus*, and the degree of plasmid loss and transposition achieved.

The potential application of this technique to studies of the molecular biology of *Xenorhabdus* is discussed.

EPIZOOTIOLOGY: THE FUTURE OF RELEASED ORGANISMS

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During the past three or four years there has been a number of meetings to explore questions relating to events which could follow the environmental release of genetically engineered microorganisms (GEMs) or, indeed, any transgenic organism whether micro or macro. A recurrent theme has been the lament that we know so little about the natural ecology of wild-type microorganisms that prediction of the fate of GEMs, or assessment of the risk such releases conceivably present, is virtually impossible. This, it is appreciated, is particularly true of free-living bacteria, fungi and Protozoa but is probably less so for human and animal diseases and also for "assessment of the transport of plant pathogens" (Alexander, 1988). The salient ecological questions on which risk assessors would ideally require information are as follows. Persistence: which determines the durability of effect. Growth in numbers: which affects the scale of impact. Dispersality: which determines the area of effect. Competitiveness: which affects the capacity to perturb microbial communities. Genetic stability: the potential for the acquisition of the engineered gene by other organisms as well as the tendency of the GEM to revert.

Until a comparison of the ecological significance of parent type, clone and GEM can satisfactorily be achieved we must doubt if many releases will be permitted. The purpose of this paper, therefore, is to suggest approaches to this problem in the context of epizootiology.

In May 1986 a key meeting entitled "Basic Research Needs in Microbial Ecology for the Era of Genetic Engineering" (Regal *et al*, 1987) took place at Scottsdale, Arizona. The meeting incorporated a workgroup to discuss the question of "the status and prospects for the development of a body of predictive (ecological) concepts". One of the specific needs identified was that new basic knowledge is required on "microbial population dynamics and adaptation (and their molecular basis), the adaptive potential and limits of clones and species in nature" and that, *inter alia*, progress could be made via the establishment of interdisciplinary graduate programmes in microbial ecology; the most important single action would be the establishment of regional centres for interdisciplinary research and training on microbial ecology. These requirements seem to have been little noted but they concern us greatly when considering the possibilities of following analytically the complex route towards the goal of assessing the risk of any individual release proposal.

From the report of the Concepts Workgroup, which pointed out that "In the past, research in microbial ecology has not focused on population dynamics with natural microbial communities", I pick out a number of questions which could apply as much to insect pathogens as to other microorganisms.

1. "The kinetics of growth of natural populations of microorganisms has proven difficult to study. However it is crucial to understand these kinetics in order to predict the competitiveness of introduced organisms to natural communities".

2. "The dynamics of effective dispersal of microorganisms is poorly understood".

3. "Survival under no-growth conditions" (persistence).

It is cheering to be able to report that for one group of microorganisms, notably the nuclear polyhedrosis viruses (NPVs), family Baculoviridae which are obligate parasites of Arthropoda and especially insects, much information has been generated on the above questions. In this paper I describe the structure of NPV epizootics in insects.

Epizootics are cyclical events showing closely interrelated temporal and spatial changes in disease incidence. Temporal infection develops in lag phase with host population increase, expressing the temporal wave form well known in the outbreak of other animal and of plant disease. NPV populations can be computed and related to host populations to interpret and predict wave dynamics. Spatial movement of disease from an epicentre embodies two readily identifiable phases: in the primary phase the log of the distance of spread (x) and log of disease proportion (y) is linear and this primary dispersal gradient (-b) has a unique value in any host-virus system. A dispersal wave next evolves, also with system-specific parameters. Thus spatial spread can be predicted for wild type NPV diseases. The interdependence of spatial and temporal change can be demonstrated in an ultimately predictive single model. Spatio-temporal fluctuations are mediated by, and governed by, values attributed to persistence and dispersal mechanisms. Short term, immediately relevant, persistence usually occurs on insect host plants, very long term persistence is in soil. Predators and scavengers ingest and excrete active NPV: this, and contamination in parasitoids, contributes strongly to dispersal. All these can be quantified in a spatio-temporal context.

How can such information assist in assessing questions raised by the proposed release of GEMs? A central dilemma associated with GEM release is that risk assessment can be effected only by following an actual release: but this may be inadmissible and may be blocked by regulating bodies requiring much more advance information. This situation strongly indicates the development of the use of severely contained ecosystems (SCEs). The function of SCEs is to compare, as isolated environmental components, wild parent type NPVs with clones and clones genetically engineered. With the proviso that the rates and patterns of field dispersal can never be duplicated in SCEs almost every other question may be amenable to valuable simulation studies. Questions should first be sharply defined. For this it is necessary to analyse possible patterns of environmental flow of NPVs (Entwistle *et al*, 1988). SCEs can then be purpose designed and controlled to address such questions as temporal rates of growth, the influence of abiotic and biotic factors on the environmental distribution of NPVs, persistence under varied environmental conditions and density dependent interactions. [For the importance of comparative host range studies see Cory & Entwistle (1988)]. Following demonstration in the laboratory that there can be genetic recombination between different NPVs, SCEs may be useable to quantify its 'natural' environmental incidence.

References

ALEXANDER, M. (1988). A microbial ecologist looks once again at risk analyses. In: Risk assessment for deliberate releases, Ed. W. Klingmüller, pp. 1-9, Springer-Verlag, Berlin.

CORY, J.S., & ENTWISTLE, P.F. (1988). Host-range testing and risk assessment for the release of genetically manipulated baculoviruses.

ENTWISTLE, P.F., CORY, J.S., & DOYLE, C. (1988). An overview of insect-baculovirus ecology as a background to field release of a genetically manipulated nuclear polyhedrosis virus. In: Risk assessment for deliberate releases, Ed. W. Klingmüller, pp. 72-80, Springer-Verlag, Berlin.

REGAL, P.J., KLUG, M., SAYLER, G., SHAPIRO, J. & TIEDJE, J. (1987). Basic research needs in microbial ecology for the era of genetic engineering (unpublished).

USE OF *GALLERIA MELLONELLA* LARVAE AS A LABORATORY MODEL TO STUDY THE FATE OF MANIPULATED BACULOVIRUSES IN COMPETITION WITH WILD BACULOVIRUSES

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Since the first observations,in the early eighties, of natural genetic recombination between closely related isolates of baculoviruses in larvae or in insect cell culture, a large utilization of DNA exchanges between these viruses has taken place in more and more laboratories (Smith & Summers, 1980; Summers, *et al*, 1980; Croizier, *et al*, 1980; Croizier & Quiot, 1981). The tremendous development of viral vectors to produce foreign proteins in insect cells infected with engineered baculoviruses lay in homologous recombination to transfer the foreign DNA sequence from a transplacement plasmid to the wild baculovirus DNA genome (Smith, *et al*, 1983,a,b; Luckow & Summers, 1988).

Galleria mellonella larvae and the MNPVs of *Autographa californica* group (*A. californica* MNPV, *G. mellonella* MNPV, *Rachiplusia ou* MNPV, *Trichoplusia ni* MNPV) have played a prominent part in the field of natural or forced recombination between wild viruses (Croizier, *et al*, 1988). Another step forward has recently been taken to study the fate of recombinant baculovirus with this biological material (Gonnet & Devauchelle, 1987; Croizier, *et al*, 1987; Vlak, *et al*, 1988). Two examples are presented to illustrate the point.

- Competition between a recombinant *Galleria mellonella* MNPV and a wild GmMNPV.

The bacterial gene NPTII (Aminoglycoside phosphotransferase II) of resistance to neomycin has been introduced into the *Galleria mellonella* MNPV (GmMNPV) genome downstream of the p10 promoter. The recombinant virus (Neo^R MNPV) forms polyhedrons. To assess the ability of Neo^R MNPV to survive competition in natural conditions a double infection with both GmMNPV and Neo^R MNPV was done. The Neo^R MNPV progeny multiplied more efficiently than the wild GmMNPV progeny. This result shows that Neo^R MNPV is apparently well fitted to competition with wild viruses, at least in the laboratory. Moreover recombinant baculoviruses with foreign genes under the control of the p10 promoter seem promising as a means of producing improved baculoviruses for biological control.

- Search for transfer of a foreign gene from a recombinant baculovirus to a closely related wild baculovirus.

In one experiment the NPTII gene introduced in Neo^R MNPV has been transmitted to R.ou MNPV by forced recombination. By cotransfection of R.ou MNPV DNA and the four SmaI restriction fragments of Neo^R MNPV genome into *G. mellonella* larvae, 0.1 to 0.2% of the R.ou MNPV progeny contained the NPTII gene. This transfer of gene from GmMNPV to R.ou MNPV occurred without any intentional selection with antiobiotics (Neomycin, Kanamycin, G 418).

In conclusion, *G. mellonella* that is easily reared and maintained in the laboratory, can serve as a convenient model to study, in a safe situation, the extent of genetic exchanges that take place between baculoviruses in many situations more or less close to those prevalent in the nature.

References

CROIZIER, G., GODSE, D., VLAK, J., 1980. Sélection de types viraux dans les infections doubles à baculovirus chez les larves de Lépidoptères. C.R. Acad. Sci., D, 290-579-582.

CROIZIER, G., QUIOT, J.M., 1981. Obtention and analysis of two genetic recombinants of baculoviruses of Lepidoptera, *Autographa californica* Speyer and *Galleria mellonella* L. Ann. Virol. 1322 E,3-18.

CROIZIER, G., CROIZIER, L., QUIOT, J.M., LERECLUS, D., 1988. Recombination of *Autographa californica* and *Rachiplusia ou* nuclear polyhedrosis baculoviruses in larvae of the lepidopter *Galleria mellonella* L. J. Gen. Virol. 69,177-185.

CROIZIER, G., GONNET, P. DEVAUCHELLE, G., 1987. Localisation cytologique de la protéine P10 du baculovirus de la polyédrose nucléaire du Lépidoptère *Galleria mellonella* L. C.R. Acad. Sci. Sér. III, 305, 677-681.

GONNET, P., DEVAUCHELLE, G., 1987. Obtention par recombinaison dans le géne du polypeptide p10 d'un Baculovirus exprimant le géne de réseistance à la néomycine dans les cellules d'insecte. C.R. Acad. Sci. Sér. III, 305,111-114.

LUCKOW, V.A., SUMMERS, M.D., 1988. Trends in the development of baculovirus expression vectors. Biotechnology 6,47-55.

SMITH, G.E., SUMMERS, M.D., 1980. Restriction map of *Rachiplusia ou* and *Rachiplusia ou-Autographa californica* baculovirus recombinants J. Virol. 33, 311-319.

SMITH, G.E., FRASER, M.J., SUMMERS, M.D., 1983a. Molecular engineering of *Autographa californica* nuclear polyhedrosis virus genome deletion mutations within the polyhedrin gene. J. Virol., 46,584-593.

SMITH, G.E., SUMMERS, M.D., FRASER, M.J., 1983b. Production of a human Beta interferon in insect cells infected with a baculovirus expression vector. Mol. Cell. Biol. 3, 2156-2165.

SUMMERS, M.D., SMITH, G.E., KNELL, J.D., BURAND, J.P., 1980. Physical maps of *Autographa californica* and *Rachiplusia* ou nueclear polyhedrosis virus recombinants. J. Virol. 34, 693-703.

VLAK, J.M., KLIKENBERG, A.F., ZAAL, K.J.M., USMANY, M., KLING-ROODE, E.C., GEERVLIET, J.B.F., ROOSIEN, J., VAN LENT, J.W.M., 1988. Functional studies on the p10 gene of *Autographa californica* nuclear polyhedrosis virus using a recombinant expressing a p10-beta-galactosidase fusion gene. J. gen. Virol. 69,765-776.

QUANTITATIVE ASSESSMENT OF MBMNPV IN A MODEL-ECOSYSTEM

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Due to recent advances in genetic engineering, some properties of baculoviruses which are used in biological pest control can now be improved. The research is directed mainly towards the engineering of viruses for increased virulence and changes in host range and field persistence.

To assess the potential risk of the release of such a modified baculovirus, a contained model-ecosystem has been developed, in which the conditions (wind, rain, sunlight) in a 0.6 x 1.20 m part of a cabbage field can be imitated and the field release of the virus can be simulated.

In a first series of experiments the ecosystem was tested using a nonmodified baculovirus, the nucleopolyhedrosis virus of the cabbage moth, *Mamestra brassicae* (MbMNPV).

The investigations were carried out to measure the dissemination and transmission of the virus in such a laboratory ecosystem, containing soil, plants, first instar larvae of the cabbage moth, earthworms and/or adults of the parasitoid, *Microplitis mediator* (Hymenoptera: Braconidae).

In the first experiment, where larvae of the cabbage moth and earthworms were introduced into the ecosystem, the virus was applied at a simple dose of 4×10^7 polyhedra (corresponding to 10^{12} polyhedra per hectare). One month later, the concentrations of polyhedra on the plants, in the soil and in the infiltration water were determined. For this, polyhedra were recovered from the plants by washing, and extracted from soil samples by a modified procedure outlined by EVANS, H.F., BISHOP, J.M. and PAGE, E.A., J. Invert. Pathol., 35, 1-8, 1980. Aliquots of the final soil and plant extracts were mixed into artificial diet and bioassayed against first instar larvae of the cabbage moth.

The results of the virus quantification showed that the overall virus amount in the ecosystem increased by a factor of more than 1000 as compared to the original inoculum. Ten percent of the virus was found on the leaves, 90% in the soil and none in the drainage. The finding that no viral activity was observed from bioassayed drainage water, in spite of the activity of the earthworms, emphasizes very well that soil can act as an efficient filter for baculovirus retention.

In a further series of experiments in the ecosystem, the concentration of the virus spray inoculum was modified ($4x\ 10^8$, 4×10^7 and 2×10^7 polyhedra per trial). It was interesting to see that the quantity of virus on the plants one month after the treatment was negatively correlated to the amount of virus applied, i.e. the higher the initial virus input, the lower the virus output at the end of the experiment. This can be explained by the fact that a high virus dose kills the larvae more quickly so that the larvae die at an early instar and contain little virus.

A very similar effect was observed in two later experiments, where we were interested in the transmission of the virus by beneficial insects and

larval migration. In these experiments, only one half of the cabbage plants in the containment were treated. In the first experiment (A), larval migration from the treated into the untreated part was prevented by a barrier of tangle foot glue. In experiment (B), the larvae were allowed to move freely between the two compartments and wind and rain were simulated. An additional, but unintentional factor in both experiments was a massive attack of aphids on all plants.

As a measure of the efficacy of the treatment, virus mortality of the larvae in the ecosystem was recorded. As shown in Table 1, virus transmission occurred in both experiments but to a very different extent.

Table 1: Virus amount on plants in relation to virus mortality in the larval population.

Experiment A			Experiment B	
Virus amount (polyhedra)	Larval mortality (%)	compartment	Virus amount (polyhedra)	Larval mortality (%)
$1,6x10^{10}$	91	treated	$1,2x10^{9}$	100
$1,6x10^{10}$	33	untreated	$4,0x10^{11}$	93

In experiment A, where only flying insects (parasites and aphids) were responsible for virus transmission, only 33% of the cabbage looper larvae released on the nontreated foliage died. More than 93% mortality was observed in experiment B, where larvae could migrate from the treated into the untreated part.

In the latter, the larvae died again at a late stage, so that even if there were few of them, the overall virus amount on the plants was the same or even bigger than in the treated part.

How far the adults of the parasite *Microplitis mediator* may play a role in the transmission of viruses of their host could not be specified in this experiment. First results obtained from laboratory tests revealed no correlation between parasitism and virus incidence.

The experiments performed in the contained ecosystem have demonstrated that a carefully timed virus release at a high dose can result in a significantly lower virus load on the plants as compared to a spontaneous occurrence of a virus disease.

After these preliminary tests using an unmodified baculovirus, trials with a genetically engineered virus are being initiated.

HOST RANGE TESTING AND RISK ASSESSMENT FOR THE RELEASE OF GENETICALLY MANIPULATED BACULOVIRUSES

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Genetic engineering is progressing rapidly in the race to produce 'improved' organisms. However, before they can be safely released into the environment any risks that may be attached to doing so must be assessed. This relies on detailed knowledge of how the organism interacts with its environment and, unfortunately, this type of study is lagging behind. At the Institute of Virology we are involved in devising a risk assessment programme for the future release of genetically manipulated baculoviruses as 'improved' control agents. Although there is already a certain amount of information on baculovirus ecology it cannot be considered exhaustive enough to enable accurate predictions to be made about the fate of a virus when it is released into the wild. Consequently it was decided early on in the project that meaningful risk assessment studies could only be carried out against a thorough background knowledge of the wild type virus:host system. This paper focuses on one small part of this investigation and one that can be readily addressed in the laboratory, namely virus specificity.

Risks in this situation are taken to be the possibility of creating epizootics in non-target species and the movement of 'foreign' genes into unintended micro-organisms. Detailed knowledge of a virus' host range is of obvious relevance when considering these two areas. There are cross-infection data on baculoviruses but wide ranging studies are rare. It is important that the progeny virus is identified to confirm true cross-infection as there is always the possibility of contamination or production of latent virus. For example, in recent host range studies on the nuclear polyhedrosis virus (NPV) of the cabbage moth *Mamestra brassicae* (Noctuidae) six species produced a virus which was not *M. brassicae* NPV. Given these provisos, the general tendency appears to be that the lepidopteran NPVs have a wider host range than the hymenopteran NPVs, granulosis viruses (GVs) and the non-occluded viruses. *Autographa californica* (Noctuidae) NPV is the most frequently quoted example with over 40 susceptible species in 11 lepidopteran families (although progeny virus was not always identified). However, even this has exceptions; recent results from host range testing on the brown tail moth *Euproctis chrysorrhoea* (Lymantriidae) NPV on 66 species from 13 families has failed to find another permissive species, including the congeneric *E. similis*. There are also indications that baculoviruses are not necessarily going to be most infective to the closest taxonomically related species. Without further broad-based host range investigations it would be unwise to predict which species are going to be cross-infected by baculoviruses.

How should we approach host range testing for risk assessment? Questions which have to be answered include:

- How many species should be tested? -
- What species should be tested? - (closely related species, species of conservation value, beneficial insects, pest species?)
- What level of susceptibility is important?
- Do we need to account for variability within and between populations?

- The viral control agent will be released in many different areas, should we host range test for each ecosystem?
- How important is the stressing out of latent virus in insect populations?

Wild virus isolates are frequently mixtures of genotypically distinct strains which could vary in host range; engineered viruses must be clones. Cloning and cell culture could thus decrease the number of hosts or affect virulence and one genetic construct is unlikely to behave like another. It will therefore be necessary to assess host range for wild type, cloned and manipulated virus.

Assuming we answer these questions and finally obtain the results, we then have the problem of interpreting them. In baculoviruses the foreign gene is incorporated in the genome and should only be expressed in insects which are normally susceptible to the virus, so host range should not expand. Engineering baculoviruses to contain foreign genes is still in the early stages and so it is difficult to predict what changes will be feasible. However, it is likely that pathogenicity and speed of kill will increase. Returning to the original perceived risks; is the death of a non target species in a localised area likely to matter? The environmental consequences of alternative methods of control are still likely to be far greater. A wider host range may also make the virus more commercially attractive. A key factor here is the nature of epizootic and enzootic virus; why do some viruses produce epizootics and others not? If a species has its own virus which does not appear to cause epizootics then a heterologous (wild type) virus, which should be less infective, is unlikely to cause problems. However, if the pathogenicity of this virus is increased or altered in some other way the outcome may well be different. More detailed research is badly needed on the nature of epizootic and enzootic viruses, looking at switching from one state to another and, in particular, assessing the role that virus pathogenicity plays in the virus:host relationship. Movement of foreign genetic material into unintended micro-organisms probably poses a greater area of concern. The likelihood of recombination with other micro-organisms should be assessed thoroughly in the laboratory before any release. Data on this area are as yet sparse, although recombination between all but the most closely related viruses is unlikely. However it is of obvious importance to address this issue carefully. With a larger number of potential hosts the possibility for multiplication (although probably less than for the wild type) and dispersal will be increased, and this will increase opportunities for recombination.

Is it possible at this stage to draw up guidelines for risk assessment studies (in baculoviruses) and for host range testing in particular? The realistic answer is probably no. Better information on host ranges and the effect of laboratory manipulation is needed before key species or groups can be recommended for what will become routine risk assessment of an ever increasing number of genetically engineered micro-organisms. Thorough host range testing is time-consuming and thus expensive and it is important that suitable protocols are decided upon which are both meaningful and manageable. How these data should be interpreted and whether the results are a cause for concern will depend very much on increasing our knowledge of virus epizootiology in a wide range of hosts and environments.

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MICROEVOLUTIONARY CHANGE IN NOVEL INSECT-BACULOVIRUS INTERACTIONS

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When a pathogen is introduced to control a pest, it is necessary to know what evolutionary and co-evolutionary changes are likely to occur, in order to predict the long-term efficacy of the measure and the long-term outcome of the interaction. Here this question is examined (a) empirically, using data for the Indian meal moth, *Plodia interpunctella*, and its granulosis virus (GV), and comparing these with data from previous, related work, and (b) theoretically, firstly for interactions which, like the present data, are novel in the sense that the pest and its own baculovirus are brought into more frequent contact with one another ("new frequency"), and secondly for "truly novel" interactions in which a baculovirus (natural or engineered) is brought into contact with a potential host for the first time.

P. interpunctella was subjected to ten generations of selection at a high virus dose (high insect mortality). Each new generation was compared to an unselected control for changes in resistance (mostly by bioassay), and several were compared to the control for 'ecological' parameters such as size and rate of development. The GV was subjected to six generations of selection at a low virus dose (very low insect mortality) in order to select for a more virulent strain of virus. Virus was extracted from the rare infected insects each generation and the virulence compared with that expected from the original strain. The various virus strains are also currently being compared genetically (by analysis of restriction fragments).

The GV showed no evidence of altered virulence. This concurs with the results of previous studies (based on repeated passage, not selection) which did however find evidence of genetic changes. The insect certainly showed no evidence of increased resistance, and may have become more susceptible. However, the selected strain showed a significantly enhanced rate of development compared to the control, indicating that a selection process had been in operation. Some previous studies have been similarly unable to select for increased resistance, but others have shown varying degrees of success.

The theoretical investigations are modifications of those by Anderson & May (1981, Phil. Trans. Roy. Soc., B.291) and Parker (1985, in 'Behavioural Ecology', Smith & Sibly, eds., Blackwell Scientific). They suggest that baculovirus virulence is likely to be at, or close to, a maximum, irrespective of environmental circumstances, whereas host resistance is likely to be much more sensitive to environmental circumstances, increasing in particular with increases in virus virulence and/or abundance. In other words, there appears to be a fundamental asymmetry between the baculovirus and host. This suggests the following conclusions.

(a) In experiments like the one reported here, the lack of response by the virus is perhaps to be expected, but an increase in host resistance was also to be expected, assuming (i) that the hosts have the biological capacity to evolve resistance and (ii) that the necessary genetic variation was present. The former is perhaps questionable. The latter is currently being investigated as an explanation for the experimental results.

(b) In "new frequency" interactions in the field, it is most likely that the balance will shift towards an increase in pest resistance to the baculovirus.

(c) In "truly novel" interactions in the field, there is likely to be an initial period of no net change while the rates of increased virus virulence and increased host resistance are approximately equal. Thereafter, however, the balance is likely once again to shift towards the host.

3. ROUND TABLE DISCUSSIONS

LIMITS AND SIDE EFFECTS OF THE CHEMICAL CONTROL OF SOIL INSECTS AND NECESSITY OF BIOLOGICAL CONTROL

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Among the insecticides used in Italy the greatest amount is for soil treatment.

Recently an investigation was carried out at the Institute of Entomology, University of Padua, in an area equal to more than 4% of that planted with maize in 1988, on the use and effectiveness of soil insecticides. It was shown that more than 50% of the insecticides (considered as commercial products) used in the Veneto region are of the soil type. However, this incidence is actually 8-9% in terms of the active ingredient distributed due to the low concentrations in the formulations (3-7%). They are mostly used on maize and beet for protecting the seeds and plants during the initial stages of development. No economical evaluations of their usefulness have been made.

On the basis of research carried out over the last 10 years in north east Italy it was found that soil insecticides have never significantly increased production of either maize or beet. Table 1 shows unpublished data concerning maize cultivation during the past years on both insecticide treated and untreated soils.

In no case was production significantly increased. On the contrary, the most widely used insecticide (phorate) was found to be phytotoxic, slowing down germination, on all the farms, to such a degree that it became statistically significant. Other insecticides also showed phytotoxicity.

On the basis of the investigation carried out on farms (each individually contacted by competent technicians) it was observed that, during the period 1983 to 1988, only about 1% of the area had to be sown again because of cut worm damage and 0.1% due to wire worm. There was no difference between farms using soil insecticides and those not.

In addition numerous farms had stopped using soil insecticides years before. Other side effects are:

- harmful to health: the use of soil insecticides while sowing seeds has caused illness in farm workers, at times even poisoning (documentable);

- water and soil pollution: though the more persistent chloroderivatives have been eliminated, carbamides and organic phosphates are present.

Effective biological products are needed to control soil insects. This is not only for ecological reasons, which are becoming more and more important, but also for strictly technical ones because of the poor reliability of chemical products in a substrate such as soil. Particular hope is placed on the entomoparasitic nematodes of the *Heterorhabditis* species which, in the laboratory, have shown promising results against wire worm larvae.

TABLE 1. The effect of soil insecticides, in different parts of Veneto, on plant density and yield of maize. On the whole in the trials the negative side effects (in particular phytotoxicity) prevail to such an extent that use of the products besides being needless may even be harmful.

EXPERIMENTAL CONDITION					PLANTS/SQUARE METER				
AREA	SOIL	RISK LEVEL	SOIL INSECTIC.	QUANTITY (kg/ha)	3 LEAVES INSECTICIDES NO	3 LEAVES INSECTICIDES YES	6-10 LEAVES INSECTICIDES NO	6-10 LEAVES INSECTICIDES YES	(TON 15%
BREDA	SANDY	LOW	PHORATE	10	5.3^{A}	4.6^{B}	5.2^{A}	5.2^{A}	n.r
S.URBANO	LOAM	LOW	PHORATE	15	6.3^{A}	3.9^{B}	6.7^{A}	4.9^{B}	13.2
PERNUMIA	SANDY	LOW	PHORATE	10	6.0^{A}	5.4^{B}	5.8^{A}	5.5^{A}	n.r.
S.DONA'	CLAY	LOW	PHORATE	10	6.6^{A}	6.2^{A}	6.2^{A}	6.2^{A}	n.r.
VILLORBA	LOAM	LOW	DIAZINON	10	5.8^{A}	5.4^{A}	5.4^{A}	6.0^{A}	n.r.
VILLORBA	LOAM	LOW	DIAZINON	10	5.7^{A}	5.6^{A}	5.7^{A}	5.5^{A}	n.r.
S.URBANO	LOAM	LOW	DIAZINON	15	6.6^{A}	5.8^{B}	6.9^{A}	6.4^{A}	13.2
AGUGLIARO	CLAY	LOW	DIAZINON	15	5.5^{A}	5.5^{A}	5.4^{A}	5.4^{A}	n.r.
S.DONA'	LOAM	LOW	BENFURACARB	12	6.2^{A}	5.9^{A}	6.0^{A}	5.9^{A}	n.r.
AGUGLIARO	CLAY	LOW	CHLORPYRIFOS	15	5.5^{A}	5.3^{A}	5.5^{A}	5.3^{A}	n.r.
CAORLE	LOAM	LOW	FONOFOS	10	5.3^{A}	5.3^{A}	5.2^{A}	5.4^{A}	n.r.
ERACLEA	LOAM	LOW	PHOXIM	10	6.5^{A}	6.2^{A}	6.4^{A}	6.3^{A}	n.r.
TORRE M.	CLAY	HIGH	BENFURACARB	10	5.7^{A}	6.0^{A}	5.5^{A}	6.0^{B}	10.5
TORRE M.	CLAY	HIGH	BENFURACARB	10	5.9^{A}	6.0^{A}	5.8^{A}	6.0^{A}	14.0
ERACLEA	SANDY	HIGH	BENFURACARB	10	6.4^{A}	6.9^{B}	6.3^{A}	6.8^{B}	9.8

HIGH LEVEL RISK - previous crop alfalfa meadow

LOW LEVEL RISK - previous crop maize or soybean

Means marked by different letters differ at p = 0.05.

METARHIZIUM ANISOPLIAE: A PROMISING BIOLOGICAL CONTROL AGENT FOR BLACK VINE WEEVIL, *OTIORHYNCHUS SULCATUS*

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The black vine weevil, *Otiorhynchus sulcatus*, is currently controlled with the organochlorine insecticide aldrin. This material is persistent, providing prolonged weevil control but additionally causing the insecticide to receive close scrutiny by the regulatory authorities. In 1991 it will become illegal to sell aldrin in the UK and in 1993 its use will be banned. There are currently no suitable alternative insecticides and there is an urgent need for new methods of weevil control.

A laboratory bioassay system has been used to examine the virulence of *Metarhizium anisopliae* isolates to *O. sulcatus* larvae at 10, 15 and 20°C and 101-82 was highly virulent at 20°C but not at 10°C. In contrast, *M. anisopliae* (275-86) was able to kill larvae at 5 10, 15 and 20°C though larval death was delayed at the lower temperatures.

In a glasshouse experiment, three *M. anisopliae* isolates gave over 80% reduction of weevil populations on *Begonia* after application of 25 ml of a conidial suspension containing 10^7 conidia per ml. Prophylactic applications of conidia completely eliminated weevils from *Begonia* while delayed applications, made 2, 4, 6 or 8 weeks after weevil infestation, gave 92, 92, 84 and 65% reductions respectively.

On outdoor-grown H.O.N.S. one isolate of *M. anisopliae* provided 62% reductions of weevil populations on *Skimmia* and 43% reductions on *Viburnum*. On outdoor strawberries, application of *M. anisopliae* to pots provided a maximum of 95% weevil control where plants were grown in field soil, but only 50% reductions in peat.

Three isolates of *M. anisopliae* survived well for 4 months in a peat compost and 16 week old conidia provided similar levels of weevil control to fresh spores.

The implications of these results will be discussed.

CONTROL OF VINE WEEVIL LARVAE ON CYCLAMEN WITH INSECT-PARASITIC NEMATODES

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Aldrin is widely used to prevent vine weevil larvae (*Otiorhynchus sulcatus*) infestations in cyclamen. Alternatives to this highly toxic organochlorine insecticide are required following announcement of its withdrawal from sale in 1990. One option is biological control using insect-parasitic nematodes. Four glasshouse trials using British isolates of *Steinernema bibionis* and *Heterorhabditis* sp. are reported here.

1. Nematode dose and application time

Four doses of *S. bibionis* (100,000, 50,000, 25,000 or 12,500 nematodes/pot) were drenched into vine weevil infested cyclamen. In some treatments the nematode doses were split and applied in two or three lots. Six vine weevil eggs/pot were applied at weeks 0 and 6. The trial was assessed after 12 weeks. One hundred percent control of vine weevil larvae was achieved by applying 25,000 nematodes/pot at weeks five and nine.

2. Application techniques

This trial tested whether nematodes sprayed onto cyclamen penetrated the shoot cover in sufficient numbers to control vine weevil larvae, and whether the control was comparable with drench treated plants. Six doses of *S. bibionis* (100,000 to 3,125/pot) were spray or drench applied to vine weevil infested cyclamen. Drench treated cyclamen had significantly less vine weevil larvae/pot than spray treated plants.

3. Curative control

S. bibionis and *Heterorhabditis* sp. were compared for their curative control of vine weevil larvae on cyclamen. *Heterorhabditis* sp. treated plants had significantly less vine weevil/pot than *S. bibionis* treated plants.

4. Preventative control

S. bibionis or *Heterorhabditis* sp. were applied (50,000/pot) to cyclamen. Vine weevil eggs were then applied at 0, 4, 8 or 12 weeks after the nematodes. Eight weeks after the eggs were applied, the plants were assessed. *S. bibionis* persisted in the compost for longer than *Heterorhabditis* sp. to give improved preventative control of vine weevil larvae.

TRIALS IN SWITZERLAND TO CONTROL THE LARVAE OF THE COCKCHAFER (*MELOLONTHA MELOLONTHA* L.) WITH THE FUNGUS *BEAUVERIA BRONGNIARTII* (SACC.) PETCH

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Introduction

In the region of our experiments (north eastern Switzerland) the development of the cockchafer is synchronous and lasts 3 years. The adults swarm between the end of April/beginning of June. During this period they mainly concentrate at the borders of forests. After a feeding/egg maturing period of about 7-10 days the females fly back to the breeding sites and deposit their eggs in the soil at a depth of about 10-20 cm. Some of them are able to leave the soil after about 4-6 days to develop a second egg batch. The larvae hatch after 4-6 weeks. They have 3 instars and their development lasts 2 years. They feed on the roots of almost all plant species including trees; all kinds of crops can therefore be damaged.

Beauveria brongniartii is a naturally occurring fungus repeatedly observed to cause epizootics among cockchafer populations. The idea to use it as a microbial insecticide is about 100 years old, but control trials have hitherto failed. The main problem consists in introducing the inoculum to the soil, the habitat of the pest insect. Most of the previous workers poured, sprayed or dusted the spores on the surface where they became fixed and did not penetrate deep enough into the soil. The observation period was also too short to reveal significant effects. In principle there are two ways to contaminate the breeding sites: (1) Introduction of infective material by mechanical methods and (2) Introduction using the egg depositing females as vectors. The first method is suitable for protection of objects (e.g. orchards, strawberry fields etc.), the second one to regulate populations or parts of them.

In Switzerland priority has been given to the second method. First trials under controlled conditions demonstrated that the fungus can be transmitted successfully to the soil where a certain proportion of the young larvae succumbed to the disease. A relatively high non-specific mortality among the progeny of treated females was additionally observed (KELLER, Mitt. Schweiz. Ent. Ges. 51, 13-19, 1978).

Results

The first field trial was started in 1976. Using a mist blower we treated swarming beetles with blastospores at the edge of a forest. Each subsequent year we sampled at the corresponding breeding site to determine the population density and to collect individuals to examine their state of health in the laboratory. Although we successfully introduced the fungus into the population we remained sceptical but during the second generation the population collapsed. In the third and fourth generation the density remained stable at a low level (about 5-10 L2/m^2 or 1 adult/m^2) and the fungus-induced mortality was still relatively high. At the beginning of the fifth generation the situation is still unchanged. In 1982 two further sites were treated in the same manner. The densities were lower and the population decrease induced by the spore treatment less pronounced indicating a density dependent effect.

In 1985 we started a large field trial using a helicopter to spray the beetles on a forest area of 70 ha with a mean dose of 2.6 x 10^{14} spores/ha (KELLER *et al.*, Mitt. Schweiz. Ent. Ges. 59, 47-56, 1986). Five of the 14 sites with an area of 19 ha were treated twice. The first treatment increased the infection rate from 21% (5-57%) to 86.5% (40-99%), the second one from 79.1% (38-96%) to 95.5% (91-98%). After the application we found infected individuals at all treated sites although at 3 sites the percentage was low or restricted to adults. The infection rate among the L3-population increased from 2% (0-5%) before treatment to 17% (0-38%) after treatment. At the beginning of the second generation the L2-population decreased at 5 sites by a factor of 0.55, at 3 sites (the same as mentioned above) it increased by a factor of 2.66. A comparison of the length of damaged forest borders showed that the treatment reduced heavy damage from 17.8 km to 9.3 km whereas those with medium and low damage remained more or less stable. This is a further indication of a density dependent effect of this control procedure.

A second large field trial was started in 1988 with the same quantity of spore concentrate (24,000 l). The application technique was slightly modified. Instead of the time-consuming treatment used in 1985 we chose a quicker flight speed to allow a rapid treatment of the whole flight area of the beetles. The lower spore density was compensated by a higher number of treatments (up to 3 per site). However, infection rates among the treated beetles were reduced as a consequence of using this method of application. The first treatment (12 sites) increased the infection rate from 17.5% (8-33%) to 62.7% (30-89%), the second (10 sites) from 45.2% (26-89%) to 59.6% (43-89%) and the third (3 sites) from 39% (30-47%) to 85.7% (77-90%). The infection rates among the larval populations after the treatment have not yet been determined. It is interesting to compare the reproduction rate (L2/adult) in 1988 in the two experimental areas. This rate was on average 5.09 within the area of the 1985 trials and 2.15 within that of the 1988 trials, supporting the results of the pilot trials when a reduction of the fitness of the progeny of treated females was observed.

Conclusions and outlook

The available results demonstrate that the treatment of swarming beetles is a suitable method to regulate cockchafer populations and to limit the damage caused by their larvae. The method acts slowly but has a long duration. The population reducing effect is sufficient to prevent damage in meadows and arable land, but insufficient for expensive crops such as orchards. We have therefore enlarged our project and studied, in collaboration with the Federal Institute of Technology, Zurich, methods to apply infective material directly to the soil. At present the most suitable one is the application of cereal grains colonised with *Beauveria* using a sowing machine. In autumn 1988 we started several trials, results are not yet available.

ENTOMOGENOUS NEMATODES FOR THE CONTROL OF SCARAB LARVAE

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Since 1932 *Steinernema glaseri Steiner* has been considered a possible agent for the control of *Popillia japonica* (Glaser, 1932), therefore large-scale experiments were carried out against the grubs in the soil (Glaser & Farrell, 1935).

Recently Akhurst (1986) reviewed the successful activity with Rhabditid nematodes against different *Scarabaeidae* species, both in laboratory and in field trials, as, for example, with *Costelytra zealandica*, with *Dermolepida albohirtum* and *Lepidiotia frenchi* (Hitchcock, 1983) and *Ligyrius subtropicus* (Omelio & Beavers, 1983). Furthermore, successful experiments were conducted in China with *Alissonotus impressicolle* (Wang Jinxian, 1986) and in Florida with *Popillia japonica* and *Rhizotrogus maialis* (Wright, *et al*, 1988). The attention of nematode producing laboratories is at present focused on the control of white grubs. The efficacy of the entomogenous nematodes, however, is variable, depending on the pest species and also its behavioural and/or physical defence strategies (Akhurst, 1986); it is influenced by the type and moisture of the soil, presence of organic materials, etc. (Poinar, 1985). Mostly *S. glaseri* is used in the experiments, but different strains of *Heterorhabditis* spp. can give similar, if not better results. The quality of the nematodes and their symbiotic bacteria have of course primary importance (Akhurst, 1985).

In Italy, bioassays and field trials were carried out with entomogenous nematodes against *Haplidia etrusca* (a pest on hazelnut-trees with a one year life cycle) (Deseö, *et al*, 1988) and *Melolontha melolontha* (a three-year cycle strain, in pasture). The efficacy of different strains of nematode species was tested in the laboratory in peat. The grubs of *H. etrusca* proved to be susceptible to *S. glaseri*, to different strains of *S. feltiae* (syn. *Neoaplectana carpocapsae* Weiser), and *Heterorhabditis* spp. The larvae of *M. melolontha* were parasitized by *S. glaseri* and *Heterorhabditis* sp. (HI-127). Interestingly, only the first and partly the second instar larvae of the European cockchafer were killed by nematodes; older larvae lost their susceptibility.

In field experiments with *S. glaseri* and *Heterorhabditis* spp. (1 million nematodes/m^2), the mortality of *H. etrusca* was about 20% both in autumn and in spring, while it was about 40% with *M. melolontha*. The trials were carried out near Napoli and near Aosta, respectively.

The conclusions of these preliminary observations in Italy are, however, bewildering. This probably applies to all soil inhabiting insects in the field; e.g. in the same plantation where we carried out our trials with *H. etrusca*, in the following year, another species infested the soil: *Anomala juni*. Indeed, the roots of hazelnut-trees can be damaged by four scarab-species, reported only in 1988 (Bianco & Viggani, 1988). Neither the exact biology, nor the susceptibility of these insects to entomogenous nematodes are known.

As regards *M. melolontha*, these trials were influenced by the age of the larvae; i.e. field experiments could be carried out only during a limited period. However, also with this insect, the presence of other

scarab-species, or strains with different lengths of life cycles, cannot be excluded.

Consequently, it seems that good taxonomic work is needed prior to applying nematodes for the control of soil inhabiting insects.

References

AKHURST, R.J. (1986) Proc. of IVth ICIP, Veldhoven; 265-268

AKHURST, R.J. (1986) Proc. of 4th Australian Conf., NZ.; 262

BIANCO, M. & VIGGANI, G. (1988) Inf. Fitopat. 1; 39-43

DESEÖ, K.V., TARTAGLIA, A. & BARTOCCI, R. (1988) Atti Giorn. Fitopat. 2; 63-73

GLASER, R.V. (1932) N.J. Agric. 34; 21

GLASER, R.V. & FARRELL, C.C. (1935) N.J. Ent. Soc. 43; 345

HITCHCOCK, B.E. (1983) Bur. Sugar Exp. Sta. 4; 8-9

OMELIO, S. & BEAVERS, J.B. (1983) Environmn. Ent. 14; 1-5

POINAR, G.O.jr. (1985) Progress in Zoology; 95-122

WANG JINXIAN (1986) Proc. of IVth ICIP, Veldhoven; 317

WRIGHT, R.J., VILLANI, M.G. & AGUDELO-SILVA, F.J. (1988) J. Econ.Ent. 81; 152-157

IMPACT OF ENTOMOPHAGOUS NEMATODES ON THE NATURAL ARTHROPOD FAUNA

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A large number of insect species is known to be successfully infected by entomophagous nematodes in laboratory tests. Few data are available on the side effects of nematodes on non-target arthropod populations. Such side effects of indigenous and/or foreign nematode species or strains will be important for registration as biocontrol agents. In some European countries nematodes have to be registered, in others (e.g. in the Federal Republic of Germany) the release of nematodes is restricted by Nature Protection Acts.

With reference to future legislation and/or registration, we studied the occurrence of entomophagous nematodes, and entomopathogenic fungi, in the vicinity of Darmstadt in 1987/88 (Kleespies, *et al*, 1989). Soil samples were taken at about 100 sites, where nematodes had never been previously used for biocontrol. *Heterorhabditis spp.* were isolated from three samples while *Steinernema spp.* and *Diplogaster spp.* were recovered from further samples. In spite of the presence of these nematode populations, a diverse insect fauna is found in the study areas.

Nature protection agencies ask for the impacts on the natural fauna of entomophagous nematodes and arthropod species used to control pest species. To study this impact, two nematode strains (*Heterorhabditis* HL 81 and *Steinernema bibionis*) were applied to four field and forest plots near Darmstadt in 1987/88. We now compare the number of individuals and insect species inside and outside of these plots by the use of photolektors (light-tight cones with a head collecting box filled partially with a preserving agent). Persistence and spread of these nematodes are recorded by means of soil samples taken inside, and at different distances outside, the release areas. *S. bibionis* could be re-isolated for about nine months after application while *Heterorhabditis* HL 81 was found for a few weeks only. Semi-field tests are being conducted with some laboratory reared insects which usually penetrate the soil only for pupation (e.g. some moths and hoverflies).

The results of these investigations will be basic for discussions about application of foreign and/or indigenous entomophagous nematodes for biological control of pest insects. Currently, foreign species may not be used in some countries, e.g. in the FRG. On the contrary Switzerland and Austria have, since 1987, allowed the use of *Heterorhabditis heliothidis* from Australia in fields and nurseries especially for control of the black vine weevil! Future legislation, and necessary registration, in the European Community should permit biocontrol of serious pests by use of entomophagous nematode species or strains of European origin.

References

KLEESPIES, R., BATHON, H., ZIMMERMANN, G. (1989): Investigations on the natural occurrence of entomopathogenic fungi and nematodes in different soils in the surroundings of Darmstadt. Gesunde Pflanzen 41, 10

COMPATIBILITY OF HETERORHABDITID AND STEINERNEMATID NEMATODES WITH PESTICIDES

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Steinernema feltiae, S. bibionis, Heterorhabditis bacteriophora and *H. heliothidis* have been tested for compatibility with 24 fungicides, 25 herbicides and 25 insecticides. A system of testing has been worked out which takes into consideration viability, infectivity and mobility of the chemically exposed infective juveniles (J3s).

The J3s were exposed in petri dishes to 6 concentrations (10000, 5000, 2500, 1250, 625 and 312 ppm) of commercially available pesticides. Distilled water was used to dilute the chemicals; 5000 J3s were used per concentration, at a rate of 1000 J3s/ml pesticide. The effects of the exposure to the chemicals were assessed as follows:

Viability/vitality. About 300 J3s were taken from each dish and observed under a microscope after 24 and 72 hours of exposure, scoring them according to the following scale:

- : J3s (apparently) dead (motionless, straight posture, not responding to prodding);
++++ : all J3s still (mostly in a curled or coiled posture, not responding to prodding);
+++ : less than 50% J3s mobile, generally with greatly reduced and slow movements.
++ : more than 50% J3s mobile, often with reduced or slow movements;
+ : J3s only slightly affected (including those moving more actively than untreated J3s).
o : as the untreated control.

Infectivity

About 1000 J3s/concentration were separated from the chemical by repeated sedimentations (3) in distilled water. They were then transferred in 1 ml water into petri dishes whose bottom was lined with a filter paper disk, together with 3 *Galleria mellonella* larvae. Mortality of these larvae was checked on the 3rd and 6th day.

Mobility

Plastic containers were filled with moistened sand and two *G. mellonella* larvae were fixed on the sand surface at one side of the container. At the opposite side, about 1000 J3s in 1 ml of the chemical were injected on the bottom of each container (3 containers/concentration), so that the J3s were ca 4 cms from the larvae. Larval mortality was checked on the 4th and 6th day; J3s were considered mobile in replicates where at least one of the larvae had been parasitized.

All bioassays were replicated at least twice, up to four times in case of inconsistent results.

Amongst all the tested pesticides, only the following were incompatible with both *Heterorhabditis* spp and *Steinernema* spp, reducing infectivity and/or mobility of the J3s: dodine, alachlor (only at 1000-5000

ppm in the case of *Heterorhabditis* spp), parathion (only at 1000-5000 ppm in the case of *Steinernema* spp), aldicarb, methomyl, propargite (only at the highest concentrations), flubenzimine, metham sodium and phenamiphos (these last two chemicals were tested at 1250 - 39 ppm). *Heterorhabditis* spp. were also strongly affected by carbofuran, phorate, carbendazim and fentin acetate at all concentrations, and by prochloraz, linuron, terbufos and fonofos at the highest concentrations.

For most of the pesticides these results are in agreement with the data from other Authors (Kovacs, 1982; Heungens & Buysse, 1987); in previous works, however, the effects on mobility (Kovacs, 1982) or on both infectivity and mobility (Heungens & Buysse, 1987) of the J3s were not assessed. For pesticides whose effects differed markedly from those reported in the literature, the fact that different commercial products were used in the present work must be considered. Therefore, the toxicity of different formulations is currently being evaluated.

Finally, it is to be pointed out that paraquat exposed J3s showed faster movements in water, but their infectivity and mobility were negatively affected. On the other hand, some chemicals (e.g. carbosulfan in the case of *Heterorhabditis* spp) noticeably affected the behaviour of the J3s under the microscope, but no influence was observed in infectivity and mobility.

References

HEUNGENS, A., BUYSSE, G. (1987). Toxicity of several pesticides in water solution on *Heterorhabditis* nematodes. Med. Fac. Landbouww. Rijksuniv. Gent, 52 (2a), 631-638.

KOVACS, A. (1982). Sensibilitá del nematode entomopatogeno *Neoaplectana carpocapsae* Weiser ad antiparassitari. Atti Giornate Fitopatologiche, 3, 11-19.

DISTRIBUTION OF INSECT-PARASITIC NEMATODES IN THE REPUBLIC OF IRELAND

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A survey was carried out to investigate the distribution of insect-parasitic nematodes in Ireland. A total of 551 samples was collected from october 1986 to October 1987. They were collected from a variety of habitats, but predominantly tillage and pasture. The soil samples were "baited" with last instar wax moth larvae (*Galleria mellonella*) in screw-top glass jars (500 cc capacity) using 5 larvae per jar. Each sample was tested twice, at 20° and at 15°C. Insects were removed from the soil after 7 days at 20°C and after 10 days at 15°C.

Insect parasitic nematodes were found to be widely distributed in Ireland. Members of the genus *Steinernema* were recovered from 10% of the samples. A single *Heterorhabditis* was also isolated. Nematodes were present at all times of the year but were less likely to be recovered in May and June (3.6% of samples were positive). They were recovered from all habitats tested (grasslands, tillage, scrub, and woodland). Nematodes were more likely to be recovered from very sandy soils (17% of samples), peats (15%) and loams (12%) than from clays (8%) or clay loams (8%).

NATURAL AND SPECIFIC MIGRATION OF THE INSECT PARASITIC NEMATODE, *HETERORHABDITIS* SP., TOWARDS VARIOUS INSECT HOSTS

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Heterorhabditis sp. are currently used in the Netherlands for the control of the black vine weevil, *Otiorhynchus sulcatus*, in horticultural crops in glasshouses. Many more insect pests are candidates for control with these insect parasitic nematodes since the nematodes have a broad host range, are virulent and are easily mass cultured. One of the handicaps for the extension of the application of the nematodes is the lack of a suitable bioassay to estimate virulence, efficacy and the influence of environmental conditions on the nematodes.

The development of bioassays for virulence and efficacy is hampered by the complexity of this biological control agent; nematodes working together with a symbiotic bacterium. The nematode is responsible for migration, localization and penetration of the insect host and the gut wall. Symbiotic bacteria and nematodes together are responsible for the death of the host (pathogenicity). Migration and penetration are prerequisites for successful parasitization as the bacteria can only contribute to the process after the nematodes have penetrated the host. Therefore, experiments were carried out to investigate the importance of migration for the development of a bioassay.

Migration experiments were conducted in PVC cylinders (4.5 cm Ø, 9 cm high), made of six separate rings, connected with adhesive tape. The bottom of the cylinder was made of a small-petri dish, fixed to the bottom ring with synthetic modelling material. The cylinders were filled with fine sterile sand (93%<180-240 µm, approx. 220 gr/cylinder), moistened with distilled water, (8% w/w). A small petri-dish was used as a lid for each cylinder. A treatment cylinder (with an insect host at the bottom of the cylinder) and a control cylinder were always inoculated simultaneously with 2000 nematodes/cylinder in 0.5 ml water. After a certain period of time the rings were separated and the sand was rinsed in 50 ml water. The number of nematodes was estimated per ring by counting four samples of 3 ml of the rinse-water. The percentage nematodes in each ring was calculated.

The difference between normal occurring migration (control cylinder) and specific migration towards *Galleria mellonella* or *Agrotis segetum* (treatment cylinder) was highest after approx. 4 hours and was most pronounced in the first and the last ring. Therefore subsequent tests lasted 4 hours and the rinse water of ring 2 to 5 was mixed before sampling. Cylinders upside down presented the same migration patterns as upright cylinders. By rinsing the sand twice in 50 ml water the percentage recovered nematodes increased by 10%, but it did not influence the distribution of the nematodes in the cylinder. Further tests were performed in upside down cylinders and the sand was rinsed once.

The patterns of both natural migration and specific migration towards *G. mellonella* were identical for the nematode strains HL81, HF85, HFr86 and HNb87 at 20°C. HNb87 was not attracted by pupae of *Tenebrio molitor* or last instar larvae of *Otiorhynchus sulcatus*, but *G. mellonella* larvae were again very attractive.

EFFICACY OF ENTOMOGENOUS NEMATODES WITH *NEODIPRION SERTIFER* LARVAE BIOASSAYED IN DISHES AND ON THE HOSTPLANT

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Neodiprion sertifer Geoff. (NS) has been known since 1974 (Cavalcaselle, 1974) as a serious pest in Central Italy causing heavy damage on *Pinus radiata* in the area of Toscana, Lazio and Umbria. In the region of Emilia-Romagna its presence was noticed in 1977 (Pollini, 1979) and since then heavy damage has also been recorded on *Pinus sylvestris* and *P. nigra* (Baronio, *et al*, 1987) in different areas.

Chemical insecticides (carbaryl and triclorphon) are used to control this pest, notwithstanding the excellent results achieved by the NS NPV also in Italy (Baronio, *et al*, 1988). Due to the slow registration procedures it was necessary to look for an alternative, and already usable, biological control agent: e.g. entomogenous nematodes.

Materials and Methods

The following nematode species were used:

1. *Steinernema* sp.; SI-815 (isolated from prepupae of *Cephalcia arvensis* in a forest of *Picea excelsa* in "Foresta del Cansiglio" BL.).
2. *S. feltiae* (syn. *Neoaplectana carpocapsae*); SI-100.
3. *S. Kraussei* (of the courtesy of Z. Mracek, Czech.).
4. *Heterorhabditis heliothidis*; NC-19 (courtesy of J.R. Finney).
5. Distilled water as control.

Bioassay No.1. 1000 juveniles in water were soaked in two filter paper disks and put in Petri dishes. Twenty second/third instar NS larvae were placed between the disks. A small shoot of *P. sylvestris* was offered as food. The experimentation was carried out at three different temperature regimes (18, 22, 26°C) in four replicates. The number of dead larvae was recorded after 24 and 48 hours.

Bioassay No.2. was carried out with 50 cm high *P. sylvestris* trees in pots. Twenty second/third instar NS larvae were put on each plant and sprayed then with 5000 juveniles in four replicates. The trees were kept at 22°C and larval mortality checked after 48 and 120 hours.

Results

In Bioassay No.1. larvae began to die after 24 hours. After 48 hours significant differences were noticed in the parasitism rates (Table 1). The percentage of parasitism was not influenced by the temperature, therefore data show the total number of NS larvae (240) exposed to each nematode species.

Table 1. Mortality rate (%) of 2nd and 3rd instar larvae in dishes.

	After 24 hours	After 48 hours
1 :	1.25 a	100.00 c
2 :	3.75 a	100.00 c
3 :	6.25 a	21.25 a
4 :	10.00 a	41.25 b
5 :	2.50 a	5.00 a

* Numbers with different letter are significantly different with Duncan's NMR test.

In Bioassay No.2 the nematodes were not effective, in fact parasitized NS larvae were only exceptionally found.

Observations on NPV

In 1984 a NPV was isolated from a NS population in Brisighella, near Ravenna (Weiser & Deseö, 1985). Further observations were made at different localities for the presence of pathogens. In 1988, again in Brisighella, pine needles had been collected with NS eggs in spring: the hatching larvae on two needles were infected by NPV. The virus was multiplied in the laboratory in NS larvae. Infected larvae were ground and sprayed in water on the trees described in the Bioassay No.2. The dosage was 10^8 PIB/ml; 15 ml suspension was applied on each plant. The mortality of the 370 larvae was 100% within a week.

Conclusion

The parasitism rate of two *Steinernema* spp. were good in Petri dishes, but not so when spraying on the trees. Finney & Bennett showed however, that even *H. heliothidis* sprayed on the foliage were effective against NS larvae, but only when kept in closed containers for a period longer than 48 hours (Finney & Bennett, 1983). Consequently, high humidity seems indispensable to good control. Considering the extremely dry conditions in the pine plantations in the Emilia-Romagna Region, the application of entomogenous nematodes does not seem promising. As regards the control of NS pupae, the lack of susceptibility to nematodes was proved clearly (Finney & Bennett, 1983).

For the time being the most effective agent for microbial control of NS is the NPV; therefore it is hoped that the NPV isolated in Italy is as effective as the other known isolates.

References

BARONIO, P., FACCIOLI, G. & ANTROPOLI, A., (1987) Boll. Ist. Entom., Univ. di Bologna, 43; 233-240.

BARONIO, P., FACCIOLI, G. & BUTTURINI, A., (1988) Boll. Ist. Entom., Univ. di Bologna, 43; 17-24.

CAVALCASELLE, B., (1974) Cellulosa e carta 25; 27-32.

FINNEY, J.R., & BENNETT, G.F. (1983) Can. J. Zool. 61; 1177-118.

POLLINI, A., (1979) Inf. Fitopat. 29 (9); 19-21.

WEISER, J. & DESEO, K. (1985) METODI ALTERNATIVI ... 1 CONV. INTERNAZ., CESENA, 151-172.

REPRODUCTION POTENTIAL OF *STEINERNEMA* AND *HETERORHABDITIS* SPP. IN AXENIC AND MONOXENIC CULTURE WITH PHASE VARIANTS OF THEIR SYMBIOTIC BACTERIA *XENORHABDUS* SPP. AND OTHER NONSYMBIOTIC BACTERIA

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The *in vitro* reproduction potential of entomoparasitic nematodes is believed to be influenced by phase variants of their symbiotic bacteria *Xenorhabdus* spp. In order to determine the reproduction potentials of *S. feltiae* (DD-136), *S. glaseri* (NC 513), *S. bibionis* (OBS III) and *Heterorhabditis heliothidis* (NC 1), nematode eggs were surface sterilized and hatching larvae were fed with phase variants (primary and secondary form) of their symbiotic bacterium *Xenorhabdus* spp., with nonsymbiotic *Escherichia coli* or cultured axenically. From these mono- and axenic cultures single females and two males were transferred to freshly established bacteria-cultures or media (*Heterorhabditis* one hermaphrodite female). The propagation potentials of DD-136 and axenically grown NC 513 were measured by counting nematodes every three days over a period of up to 24 days. Potentials of the other strains were counted on the third and fifteenth day.

In axenic cultures only *Steinernema* spp. reproduced. There were no significant differences obtained from plates with primary- or secondary form symbionts, with the secondary form symbionts of *Steinernema* spp. even resulting in a higher maximum yield than primary form symbionts. Growth on *E. coli* resulted in a delay of development, with final nematode counts not significantly lower than when grown on symbiotic bacteria. For axenic cultures of DD-136 highest yields were on rat liver, with final counts double the size of yields on secondary form symbiont. The reproduction-potential in liquid axenic culture of strain NC 513 was comparable with yields on rat kidney, but lower than on rat liver.

The statement, that secondary form symbionts cause detrimental effects on nematodes reproduction, can no longer be maintained. Contrary results may have been influenced by polyxenic conditions or physiological changes of the nematodes.

MOSQUITO CONTROL WITH MICROBIOLOGICAL INSECTICIDES IN EUROPE AND ASIA

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Mosquitoes are frequent vectors of most important vector borne diseases of man like malaria, yellow and dengue fever, encephalitis and filariasis. These pest insects are also the reason for lowering the quality of human life by annoyance e.g. in the Upper Rhine Valley. The Rhine floods at least once a year, leaving behind thousands of puddles, ponds and ditches as breeding sites for the floodwater mosquitoes like *Aedes vexans*, the most frequent species.

The high efficacy and the oustanding environmental compatibility of *Bacillus thuringiensis israelensis (B.t.i.)* are the reasons why commercial *B.t.i*-products have been used by the *"Kommunale Aktionsgemeinschaft zur Bekämpfung der Stechmückenplage" (KABS)* in routine treatments against mosquitoes in the Upper Rhine Valley. The KABS with a yearly budget of 1.8 million DM engages about 300 workers who control mosquito larvae in an area of approximately 500 km^2 and serves about 2.5 million people.

Since 1982 more than 36,000 ha have been treated with 17,000 kg of *B.t.i.* powder and 16,000 litres of flowable concentrate. During high water levels, causing widespread inundation, about 200 tons of *B.t.i.*-sand granules were applied by helicopter. More than 200,000 *B.t.i.*-briquets were applied in the breeding sites (e.g. rainwater containers) of *Culex pipiens molestus*.

As a result of these applications the mosquito population in the Upper Rhine Valley was reduced each year by more than 95%.

The cooperation in the field of microbiological mosquito control becomes more and more important with the increasing environmental problems and the resistance of vectors against chemicals.

In the province of Hubei more than 20 million people on both sides of the Yangtsekiang River are threatened by the malaria agent *Plasmodium vivax*. During 1985 more than 140 thousand cases of malaria were reported. The major vector is the fever mosquito *Anopheles sinensis*, the control of which with insecticides has recently become more and more difficult due to the development of resistance as well as to ecological and toxicological risks. Other diseases transmitted by mosquitoes in Hubei are Japanese-B-encephalitis (transmitted by *Culex p. quinquefasciatus*, *C. tritaeniorhynchus*, *Aedes albopictus* and *A. sinensis* and also Brugian and Bancroftian filariasis transmitted mainly by *C. p. quinquefasciatus* and *A. sinensis*.

In a cooperation programme between the Province of Hubei and Baden-Württemberg the scientific and organizational prerequisites for large-scale applications of microbial agents are being created. The aims of this programme are in detail:

a) more investigations on biology and ecology of mosquito vectors of human diseases as a basis for better microbial control of mosquitoes.

b) improvement of B.t.i. - formulations with regard to better control of *Anopheles* larvae;

c) intensive exchange of practical knowledge and technology in mosquito control.

Experiments and routine treatments with different *B.t.i.* and *Bacillus sphaericus* (B.s)-preparations have been carried out in the laboratory and under field conditions specific to the Hubei Province.

In order to get a better base for biological mosquito control we investigated the specific breeding conditions of the most important mosquito species taking into consideration abiotic and biotic parameters. As a result of these investigations we can pinpoint the typical breeding sites of each important vector species for quick reference during control operations.

We use microbial agents for mosquito control because the protection and the encouragement of all natural predators is a very important maxim of our work. As the predators are not affected by microbial agents, they can continue to feed upon newly hatching mosquito larvae after treatment of the breeding sites. Fish and water bugs are the most effective predators in the mosquito breeding sites in Hubei, e.g. *Macropodus opercularis* eat more than 900 larvae of *Anopheles* or *Culex* a day.

In Indonesia, up until the present, the organophosphate insecticide Temephos has been mostly used in water containers for the control of larvae of *Aedes aegypti*, the most important vector of Dengue. Recently a sporeless form of *B.t.i.* as a tablet formulation has been tested against larvae of *A. aegypti* very successfully. These tablets can be used without risk in drinking water bodies and should be especially suitable for mosquito vector control programmes involving community participation and volunteer organisations.

A COMPARATIVE STUDY OF COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* (SAY)) SENSITIVITY TO PREPARATIONS OF *BACILLUS THURINGIENSIS* BERL. SSP. *TENEBRIONIS*

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Bacillus thuringiensis Berl. ssp. *tenebrionis* (*B.t.t*) = var. *san diego* was tested to assay its effectiveness against *Leptinotarsa decemlineata* (Say) (Colorado potato beetle, CPB) on eggplant both in field conditions and in greenhouse potted plants. A greenhouse trial on its persistence was also conducted.

The field tests on cv. *Riminese* were carried out by comparing 5 replicates of 4 treatments: (i) SAN 418 WG (1.25 g/litre), (ii) SAN 418 62 (5.8 cc/litre), (iii) SP 363 (12.5 cc/litre) and (iv) control with water only.

In each plot, four plants per row of uniform size were infested with 15 larvae for each of the four instars.

In the shaded greenhouse trials, potted plants were infested with five larvae for the first 3 instars and then treated as above. There were six replicates per treatment. The persistence trial was conducted in the same greenhouse using only treatments (i) and (iii) on first instar larvae. The plants were infested 48 and 96h post spraying. Again, six replicates of five larvae per plant were employed.

The findings of the filed trials are shown in Table 1, excluding the fourth instar data because ne effects were observed. Preparations of SAN 418 WG and SP 363 were the most effective.

Tables 2 and 3 show the data for both greenhouse trials. The two above preparations were again proved to be the most effective. The larvae in all trials died between 4 and 7 days post treatment, although they had stopped feeding immediately. Effectiveness of *B.t.t.* decreased as larval age increased.

The use of *B.t.t.* preparations is a promising tool for CPB control on eggplants. Side-effects of insecticides usually employed against CPB prevent other biocontrol strategies. Furthermore, the use of *B.t.t.* will be compatible with the releases of the egg parasitoid *Edovum puttleri* Grissell.

Table 1 - Field trial.

	Percentage of CPB larval mortality		
	1st instar	2nd instar	3rd instar
SAN 418 WG	64.0 a	35.83 ab	9.97 a
SAN 418 ISC 62	41.56 b	17.41 b	11.81 a
SP 363	73.33 a	40.77 a	16.36 a
CONTROL	9.15 c	12.77 b	10.67 a

Column values followed by the same letter are not significantly different (P<0.05, Duncan's Test)

Table 2 - Direct toxicity in a shaded greenhouse

	Percentage of CPB larval mortality		
	1st instar	2nd instar	3rd instar
SAN 418 WG	98.33 a	86.67 a	80.83 a
SAN 418 ISC 62	89.60 a	62.17 a	61.00 a
SP 363	96.67 a	80.64 a	61.00 a
CONTROL	0.00 b	4.33 b	20.50 b

Column values followed by the same letter are not significantly different (P<0.05, Duncan's Test)

Table 3 Persistence in a shaded greenhouse.

	Percentage of CPB larval mortality	
	1st instar	
	after 48 h	after 96 h
SAN 418 WG	100.00 a	86.67 a
SP 363	86.67 a	81.67 a
CONTROL	0.00 b	0.00 b

Column values followed by the same letter are not significantly different (P<0.05, Duncan's Test)

BACILLUS THURINGIENSIS PREPARATIONS FOR THE MICROBIAL CONTROL OF LEPIDOPTEROUS PESTS IN ISRAEL

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The main problems in the use of *Bacillus thuringiensis* (*B.t.*) in agriculture arise from the microbial specificity in insects, the feeding behaviour of bollworms and borers and the low *B.t.* persistence caused by plant and environmental factors. To reduce these problems, two research efforts have been undertaken: 1. Development of screening programs of *B.t.* strains against lepidopterous insects, 2. Improvements in the microbial formulations against cotton pests.

A simple potency bioassay based on neonate larvae was developed, in addition to the official bioassay using 3rd instar larvae. In these bioassays, the diets were standardized for feeding of several lepidopterous larvae. Neonate feeding was confined to petri dishes for 72 h after which mortality was recorded. A preliminary screening was conducted at a single *B.t.* concentration equal to the LC50 of the microbial reference HD-1-S-80 for insects susceptible to this *kurstaki* strain. The most active strains in this screening were selected for potency determinations.

The insects used, so far, in this program were: *Heliothis armigera*, *Earias insulana* and *Spodoptera littoralis*. In this potency screening *B.t.* var. *kurstaki* HD-263 was significantly more potent than the HD-1 strain against *H. armigera* and *E. insulana*. Also a potent var. *aizawai* strain was selected against *S. littoralis*.

Improvements in the commercial *B.t.* formulations against *H. armigera* in cotton were made by using the microbial wettable powder in granular baits. The wheat-bran based granules contained a commercial product of *B.t.* var. *kurstaki* HD-1 (16,000 IU/mg) and a protein mixture to stimulate larval feeding. The potency of the granular preparation was determined by means of the neonate bioassay. The activity and persistence of the granular *B.t.* preparation was evaluated by field bioassays on cotton leaves, flower buds and bracts. In this bioassay single early fourth instar larvae were caged on the cotton plant for three days. Mortality, larval weight and leaf consumption were recorded after three days of feeding. The activity of the microbial granules was compared with that of the *B.t.* wettable powder in an aqueous mixture. The microbial persistence was determined by exposing the *B.t.* products to field conditions for 2-3 successive days. At equal potencies, larval mortality caused by the *B.t.* granules on the leaf was 2-3 times higher than that obtained with the aqueous *B.t.* mixture. Similar differences in mortality were recorded when the microbial preparations were applied inside the flower bud. On the bract, control level by the granular formulations was lower than on the flower bud but the *B.t.* aqueous mixture has no effect on the larvae. Possibly, bollworm control in cotton could be improved by the use of granular *B.t.* baits as an alternative to the aqueous spray.

CRYOPRESERVATION OF STEINERNEMATID ENTOMOGENOUS NEMATODES

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The lack of suitable methods for the cryopreservation of Steinernematid nematodes limits the safe, cheap, long-term storage of strains and species, and their efficient use in the field for control of pests and disease vectors. Preliminary studies with a slow-cooling method, long used for cryopreservation of protozoa and infected arthropods yielded variable, generally low, survival rates for the unsheathed infective dauer (L3) stages of a range of species and strains of *Steinernema* (= *Neoaplectana*) and *Heterorhabditis* (Minter; unpublished). The outer (L2) cuticle impedes cryoprotectant penetration; exsheathment (5 mins. in 0.1% sodium hypochlorite at 22°C), followed by exposure to 35% ethanediol at 20°C for 30 mins, and snap-freezing in liquid nitrogen (LN_2), improved both survival and viability (James & Minter: unpublished).

Recent studies with the All strain of *Steinernema feltiae* (*N. carpocapsae*), [Smith *et al.*, *Cryobiology*, in press] reported here, show high rates of survival of infective stages with further technical modifications.

The following aspects were studied: 1) cryoprotectant tolerance (to dimethylsulphoxide [DMSO; Me_2SO], ethanediol, methanol & glycerol); 2) cryoprotectant exposure period and temperature; 3) slow-cooling (circa. 1°C min^{-1}) vs. snap-freezing in liquid nitrogen (LN_2 ; c. 5 100°C min^{-1}).

Good results (30-34% viability) were obtained when dauer-larvae from *in vitro* culture were exsheathed, washed twice and resuspended in water; 20 µl drops were spread over 5 x 40 mm strips of glass coverslip, incubated in 60% (v/v) methanol [or 45% v/v glycerol] at 0°C for 20 mins, followed by snap-freezing [plunged into LN_2; c. 5 100°C min^{-1}]. After thawing in warm water (22°C: c. 10 °C min^{-1}; also gives instant 1:100 cryoprotectant dilution], viability was assessed microscopically at 4 and 24 hours (4 hr results: 34 ± 15.9% for glycerol). Recent experiments, with minor modifications, improved survival and viability to about 65%. Viable larvae were capable of further *in vitro* growth and development. Methanol acts as an intracellular cryoprotectant; glycerol protects L3s by induction of partial desiccation. Nematodes are vitrified during snap-freezing.

Experiments with other cryoprotectants (ethanediol; DMSO) and cryopreservation routines (e.g. pre-cooling to -60°C at 1°C min^{-1}, followed by snap-freezing in LN_2) were less successful.

FIELD EFFICIENCY AND ENVIRONMENTAL PERSISTENCE OF THE GRANULOSIS VIRUSES OF THE SUMMER FRUIT TORTRIX, *ADOXOPHYES ORANA* F.v.R.

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The summer fruit tortrix *A. orana* is a serious pest of apples and pears. In our climate *A. orana* has two generations a year, one in the summer and another from autumn to spring with L_2-L_3 diapausing during the winter.

Microbiological control of *A. orana* was attempted with *Bacillus thuringiensis*, nuclear polyhedrosis virus (AoNPV), as well as a granulosis virus (GV) found in Japan (AoGVJ). Whereas obtaining consistently good results was not possible with *B. thuringiensis*, the costs for producing the quantities required were too high in the case of the more effective viruses.

In the late seventies a potent GV was isolated by A. Schmid in the Valais, Switzerland (AoGVS) and tested later in the laboratory. Beside describing the epizootic properties of the AoGVS, the aim of the study was to test the virus in the field and to evaluate the effect of spring applications at concentrations lower than those of the AoGVJ used in Japan. Concentrations are given as capsular inclusion bodies (CIB).

In the first year concentrations (1.7×10^{14} CIB per hectare) four times lower than used in the field experiments in Japan were applied. Because the mortality in the treated generation surpassed 95%, the concentration was further reduced to 5×10^{13} CIB per hectare, which in nine field tests caused mortalities of 80-100%.

Very good protection of the fruits was also obtained by twice applying a ten times lower concentration of the virus, that is 5×10^{12} CIB per hectare. The virus quantity required for the two applications corresponds to approximately 500 larval equivalents per hectare. The reduction in costs resulting from the lower concentration was a first step towards the commercialization of the virus and its use in the control of *A. orana* on a larger scale.

In Japan, as much as 21% natural mortality caused by GV was observed in an untreated control field. In the treated fields GV incidence in the generation following treatment was even higher. This illustrates the high tenacity of the GV under the environmental conditions in Japan, where all treatments were applied on the first of two summer generations. However, treating the summer generation does not lessen the damage, because the GV does not kill the insects until the last larval instar. Consequently, applications in Switzerland were timed for early spring, being directed against the overwintering generation. Under the climatic conditions in the Valais with two generations of *A. orana* only, early spring application - besides the direct mortality mentioned above - resulted in 20% larvae killed by GV in the summer generation and another 20% in the following spring, without counting the larvae having died in the hibernaculum. In the next two generations the virus was still traceable but could not prevent a strong increase of the *A. orana* population.

The soil was shown to be the main reservoir for the persistence of the virus. In the Valais, the soil is light and sandy and therefore viral transmission from the soil to the leaves is favoured by the heavy winds

characteristic for this region and by the mulching of the orchards. It may not be by chance only that the GV was found in the Valais, since there the conditions for longtime persistence of the virus are the best.

The AoGVS is now commercially available in Switzerland under the product name CAPEX. A provisional clearance for the treatment of 50 hectares has been obtained.

FIELD PERSISTENCE OF *SPODOPTERA LITTORALIS* NUCLEAR POLYHEDROSIS VIRUS

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A collaborative project between the ODNRI and PPRI on the use of a nuclear polyhedrosis virus (NPV) to control the cotton leafworm, *Spodoptera littoralis*, on cotton in Egypt has included an extensive study into the field persistence of the virus.

Highly purified virus was rapidly inactivated with little infective virus remaining on the cotton plant after four days exposure. The factors determining virus persistence were inactivation by sunlight and physical loss of polyhedra from the plants. Both of these effects were, however, considerably less in the shaded regions of the plant such as the lower surface of the leaves and toward the bottom of the plant canopy. No inactivation was attributed to the highly alkaline cotton leaf exudates or field temperatures over an 8 day period.

Filtering sunlight showed that wavelengths between 300 and 320 nm accounted for most of the virus inactivation. Several UV protectants were identified but no formulation was significantly more effective than unpurified virus alone; the resistance of unpurified virus to UV inactivation was attributed to the presence of insect homogenate.

Physical loss of polyhedra was attributed to the abrasive action of wind and sand and several gums and stickers were tested to counter this. None was completely effective in preventing physical loss and none of the formulations was more physically persistent than unpurified virus.

The results showed the unpurified virus persisted for several days or weeks in the shaded areas of the cotton plant where the target insect is located and therefore no further formulation was necessary to improve persistence.

BACULOVIRUS-HOST AND PARASITOID-BACULOVIRUS-HOST INTERACTIONS

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In order to use baculoviruses as microbial insecticides, especially in integrated pest management programs where they work best, consideration of all effects, other than mortality on a target larval generation, could aid baculovirus effectiveness from both a practical and an ecological point of view. To this end two aspects are of interest a) effects of baculovirus diseases on insect host physiology and b) the interactions of parasitism with baculovirus disease.

Our laboratory tests on the effects of sublethal doses of nuclear polyhedrosis virus (NPV) on *Spodoptera littoralis* demonstrated alterations of male and female reproductive capacity. Egg viability was significantly reduced when adult males or females, that had been treated as larvae, mated with untreated females or males respectively. The reasons for virus infection reducing egg viability through males is now under investigation in our laboratory.

Although in natural populations parasites are adapted to host baculovirus diseases to avoid adverse effects, interactions between parasites and baculoviruses undoubtedly would occur if both are used jointly in pest control. We are concerned with the mechanisms by which parasitized larvae are less susceptible to baculovirus than unparasitized ones.

FIELD PERSISTENCE OF *BACILLUS THURINGIENSIS* SUBSPECIES TENEBRIONIS

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In 1982, the new strain "BI 256-82" of *Bacillus thuringiensis* was isolated in the Institute of Biological Pest Control in Darmstadt. It is the first strain of *Bacillus thuringiensis* subspecies *tenebrionis* (*B.t.t.*) and represents a new pathotype C, which is only effective against the larvae of some Chrysomelidae. One of the most sensitive and important Chrysomelids is the Colorado Potato Beetle (CPB). The LD_{50} of first instar larvae of the CPB was determined to be 11000 spores and crystals, but only the crystals are important, the spores do not increase efficacy.

Since 1984, the good efficacy of *B.t.t.* against larvae of CPB has been confirmed in field trials. In 1988, three applications of *B.t.t.* were sufficient to control the larvae of CPB for the whole season.

Parallel to this field trial, the persistence of an unformulated, unpurified *B.t.t.* preparation and a formulated preparation containing hardly any spores had been tested. To investigate the persistence of the foliar deposit of *B.t.t.*, we took leaves from the treated plots, fed them to first instar larvae and noted the mortality.

In the first persistence test the formulated *B.t.t.* started with a mortality of 92% at the day of application and kept this high mortality for two days. Then it decreased, first slowly to 80% by the fourth day and then, rapidly, to 0% on the seventh day after application. The mortality of the unformulated *B.t.t.* had already decreased to 70% by the first day after application, and then was parallel to the formulated *B.t.t.*, but always one day earlier.

In the second persistence test both *B.t.t.* preparations started with a high mortality. The formulated *B.t.t.* maintained this high mortality for one day, then the mortality decreased rapidly. The mortality of the unformulated *B.t.t.* decreased at once and five days after application there was hardly any mortality in both preparations.

In addition to these field trials, we carried out a bioassay in the laboratory with leaves of potato plants grown in the glasshouse, which were sprayed with unformulated *B.t.t.*. In comparison with the results of the field trial, where the high mortality decreased after 2-3 days, the mortality of the larvae in the laboratory test stayed very high for more than seven days , before it started to decrease slowly. There was still 50% mortality after 12 days.

In order to get some ideas about climatic factors, that could be responsible for the earlier decrease in the field, we recorded the rain and the hours of sunshine during the field trial.

In the first persistence test, there was no rain and only 5.6 hours of sunshine during the first three days after application. In the second persistence test, there was a little rain just after application of *B.t.t.* and strong rain one day later. The sun shone for 20.3 hours during the first three days.

It is, therefore, possible that the earlier decrease of mortality in the second persistence test might have been caused by the rainfall and/or the higher irradiation during the first days after *B.t.t.* application.

First experiments in the laboratory to examine the influence of sunlight irradiation alone have shown that artificial solar-light also results in a decrease in mortality.

PERSISTENCE OF *BACILLUS SPHAERICUS* IN SEVERAL NATURAL CONDITIONS AND INTEGRATED MOSQUITO CONTROL PROGRAMMES

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Mosquito control programmes have, for several years, included the use of entomopathogenic bacteria, *Bacillus thuringiensis* H14 and *Bacillus sphaericus* Neide, because of the resistance to chemical insecticides developed by most vectors of human diseases.

Several authors have evidenced the persistence of these micro-organisms in the treated spots and established correlations with some environmental conditions (shade, pollution).

Furthermore, *Bacillus sphaericus* was shown to recycle by passing through the larval gut of the target *Culex pipiens* but also of some other arthropod species which are insensitive to the toxin (Karch et al 1989).

As a result, after lowering of the *C. pipiens* population with *B. sphaericus* treatment, other species may spread and become a replacement nuisance as was observed recently with *Culiseta annulata* in the south of France.

This observation suggested the combined use of bacterial and chemical agents. The aim of our study was, therefore, to investigate whether it would be suitable to mix *B. sphaericus* and the usual chemicals.

MOBILITY AND PERSISTENCE OF STEINERNEMATID AND HETERORHABDITID NEMATODES IN THE SOIL

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The mobility and persistence of *Heterorhabditis heliothidis*, *H. bacteriophora*, *Heterorhabditis* sp. (Hl 81 strain), *Steinernema feltiae* (syn. *Neoaplectana carpocapsae*) (I-100, 1192 and Mexican strains) and *S. glaseri* in the soil were studied in field conditions. The experiments were carried out simultaneously in two different soil types (type "A": clay 26% silt 62%, sand 12%; type "B": clay 58%, silt 34%, sand 8%) situated four km apart. At the time when the trials started, the "A" soil was covered with grass, while in the "B" soil a soya-bean crop was germinating. Prior to beginning the experiments, the soils were carefully checked for the presence of Rhabditid nematodes.

At each site, 6 sqm (1.5 x 4 m) plots were lined out; 4 m were left between the plots. On 15th May 1987 the nematode species/strains were applied on the surface of the plots by means of a watering pot, at the rate of 10^6 infective juveniles (J3s)/sqm. Since then, samples were taken during a 307 day long (soil "B") and a 403 day long (soil "A") period. On each sampling occasion, 6 soil samples were collected (three from within the plot, three 50 cm away from the plot) to a depth of 20-40 cm by means of a 30 mm diameter gouge auger. The samples were then divided into 4 cm pieces which were checked individually for the presence of nematodes by the "Galleria-trap" method.

All the tested nematodes showed good vertical dispersion; in fact, all of them were detected from the soil surface down to a depth of about 20 cm at the first sample (4th day). However, they differed greatly in persistence, the presence of *Steinernema* spp. decreasing very sharply. Amongst them, the longest lasting was the I-100 strain (which was found for the last time after 74 days in the "A" soil), while the others persisted 19 days at the longest. On the other hand, *H. bacteriophora*, *Heterorhabditis* sp. and *H. heliothidis* were much more persistent (403, 403 and 153 days respectively), though their presence in the soil samples became low after much shorter periods.

Apart from the 1192 strain of *S. feltiae*, all nematodes persisted longer in the "A" soil; the difference was obviously more remarkable for the longest lasting species (i.e. *H. bacteriophora* and *Heterorhabditis* sp), which were found for the last time in the "B" soil after 74 days (28th July). Interestingly, neither *H. bacteriophora* nor *Heterorhabditis* sp (Hl 81 strain) were found in both soil types on 19th November (188th day), but they were successively found again in the "A" soil (307th, 355th and 403rd day samplings).

The fact that *Heterorhabditis* spp persist much longer than *Steinernema* spp in the soil had been observed previously (Rovesti & Deseö, 1987); in those circumstances, *H. heliothidis*, *S. feltiae* "1192" and *S. glaseri* were last found after 400 (last sampling), 203 and 9-39 days respectively. However, the different period of application of the nematodes (in that case 13th October) makes any direct comparison with the present data difficult.

None of these nematodes were ever found outside the plots, in contrast with the results of Poinar & Hom (1986). Though some suppositions could be put forward to explain this fact, further work will have to be carried out to clear up such aspect.

References

POINAR, G.O. Jr., HOM, A. (1986). Survival and horizontal movement of infective stage *Neoaplectana carpocapsae* in the field. Journal of Nematology, 18(1), 34-36.

ROVESTI, L., DESEÖ, K.V. (1987). Mobility and persistence of entomoparasitic nematodes in field conditions. European Meeting on microbial control of weevils and environmental persistence of pathogens and nematodes, September 2-4, Versailles, France.

PAECILOMYCES FUMOSOROSEUS PERSISTENCE : INACTIVATION OF CONIDIA BY SIMULATED SUNLIGHT RADIATIONS

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In order to study the inactivation of fungal conidia by sunlight, laboratory experiments were carried out in illuminated incubators. The light sources were OSRAM H.Q.I. TS 400 W high-pressure metallic halogenure lamps. Inocula of fungal spores, deposited on cellulose supports, were exposed to different ranges of wavelengths using Schott filters (WG 280, WG 300 and GG 400). A combination of different filters, exposure periods and distances from the light sources to the exposure surface were used to investigate the effects of both irradiance (Wm-2) and irradiation (Jm-2) in UV-B, UV-A, visible and infrared radiations. Moreover, a specific device based on temperature regulation of plates was set up. This permitted the monitoring of temperatures on the surfaces of the inoculum supports and thus restricted the study to the photic effects of radiation. Previous experiments on conidial persistence demonstrated that the effects of visible radiation could interact with the effects of hygrometry. Consequently a second specific device was set up in order to investigate the influence of this factor.

The germination rate and the viability (colony forming units) of irradiated spores dropped very rapidly, within approximately 20 min, when exposed to radiation, including UV-B. Most mortality occurred within 100 min in the inocula exposed to radiation above 320 nm (including UV A). Within these durations of exposure, visible and near infrared radiations did not induce any lethal effects on *P. fumosoroseus* conidia. On the other hand, after 10 hrs of exposure to radiation above 400 nm, a decay of spores was noted. Thus the inoculum was entirely killed within 40 hrs. In darkness, the viability of *P. fumosoroseus* spores remained at the original level for the first 5 days and then decreased according to the humidity level.

In summary, results showed that radiation delayed the germination process. This retardation could reach 96 hrs. Furthermore, the lethal effect of each specific range of wavelengths (either UV-B or UV-A or visible and infrared) depended on the dose (JM-2). For example, the UV-B dose required to inhibit germination at 50% was half as much at 3.51 W m-2 irradiance as at 0.85 Wm-2.

A preliminary study devoted to modelling showed that lethal effects of simulated solar radiations on *Paecilomyces* conidia could be predicted using logistic models.

THE FIELD SURVIVAL OF CONIDIA OF *ERYNIA NEOAPHIDIS* AND THEIR POTENTIAL AS AN INOCULUM FOR APHID CONTROL

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The infective propagules of the Entomophthorales are the conidia but these are difficult to produce on a commercial scale and to store. Furthermore they were believed to remain infective for only a few hours or, at best, days in the field. Consequently in most programmes on the use of these fungi for biological control, other propagules, the resting spores or mycelium, are chosen for commercial exploitation.

Experiments at Rothamsted, however, suggested that conidia of *Erynia neoaphidis* were still infective for pea aphids (*Acyrthosiphon pisum*) after 7 days, often longer, on a leaf surface in the field. This was corroborated by laboratory experiments which showed that conidia on a leaf surface retained some infectivity at 18°C after 2 wk, at 50, 55 and 90% RH though for only 7 days at the intermediate humidities of 70 and 77% RH. These results were supported in tests on the survival of conidia on the surface of glass coverslips. At 20°C, survival was longest (at least 21 days) at 40% RH and was longer at 100, 90 and 55% RH than at 70 and 77% RH. Further tests defined more closely the conditions in which conidia survive longest. On a glass substrate at 40% RH and 10°C, infectivity was retained for at least 35 days.

The physiological state in which the conidia remain infective is unknown. On non-host surfaces they produce secondary and successive order conidia only so long as the humidity remains above about 97% RH. At lower humidities they fail to germinate. In our experiments the inoculum of conidia was collected from infected aphids over a 24 h period in a saturated atmosphere, quite long enough for many primary conidia to have germinated and discharged secondary ones, before transfer to the test conditions. Thus the inoculum would have comprised a mixture of primary, secondary and possibly higher order conidia. The secondary conidia are similar to but usually smaller than the primary ones and there is some evidence that they are more resistant to adverse environmental conditions.

Our results have encouraged us to reconsider the possibility of using conidia directly as an inoculum for aphid control. The results of applying conidia in suspension are always unsatisfactory probably because the natural adherence of the spore to the host is an important component in invasion and such adhesion is modified when the conidia are suspended. We are now investigating the possibility of applying conidia as they are discharged from the conidiophores. The sporulating fungus is placed in a cylinder through which moist air is passed so that as the conidia are discharged, they are caught up in the air flow and pass out through a nozzle. The nozzle is directed at the aphid infested plant. Over 60% of pea aphids submitted in this way to a few minutes application of conidia of *E. neoaphidis* were infected. While it is difficult to envisage how such a device could be operated on a field scale it may have applications in the glasshouse, gardens or horticulture.

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DIFFUSION ET VIRULENCE DE CHAMPIGNONS ENTOMOPATHOGENES EN PIEMONTE

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Les recherches jusqu'à présent conduites pour une bonne part en collaboration avec l'Institut de Entomologie agricole et Apiculture de l'Université de Torino, depuis l'année 1983, ont consideré l'isolement, l'identification, la virulence, la diffusion en zones differentes du Piemonte de champignons pathogènes pour des insectes phytophages, comme *Corythucha ciliata* Say, *Zyginidia pullula* Boh., *Sitobion avenae* (F)., *Parectopa robiniella* Clemens, *Melolontha melolontha* L., l'unique qui provient de la Vallée d'Aoste. On a même expérimenté l'action de l'inoculation en champ de mycètes entomopathogènes. Ces recherches ont été exécutées avec la contribution du C.N.R.-I.P.R.A.

C. ciliata

Les champignons entomopathogènes *Beauveria bassiana* (Bals.) Vuill., *Verticillium lecanii* (Zimm.) Viegas, *Paecilomyces farinosus* (Helm ex S.F. Gray) Brown et Smith ont été obtenus des adultes hibernants sous l'écorce de platanes de zones différentes du Piemonte.

Les trois deuteromycètes essayés en épreuves d'infection en laboratoire se sont montrés hautement virulents pour l'insecte; en essais d'infection sur adultes hibernants sur platanes de routes citoyennes, leur action a reçu l'influence de la charge d'inoculation et s'est présentée persistente au bout d'une année après les traitements.

Z. pullula

V. lecanii et *Zoophthora radicans* (Bref.) Batko se sont révelés parasites efficaces du "cicadellide". A' l'égard de l'insecte, pourtant *V. lecanii* peut même se conduire comme un mycète saprophyte. Des différents isolés de *V. lecanii*, tous provenants des exemplaires de *Z. pullula*, en épreuves d'infection ont donné, à l'égard de l'insecte, virulence très variable avec des pourcentages de mortalité totale (100%), moyenne (57-72%), contenue ou très contenue (10-43%). Ce résultat confirme qu'à l'état naturel le deuteromycète se conduit comme pathogène ou comme saprophyte.

S. avenae

L'aphide en cultures céréalières du Piemonte peut être limité par *V. lecanii*, *Fusarium oxysporum* Schlecht. emend. Snyder et Hansen, *F. tricinctum* (Corda) Sacc., *Entomophthora planchoniana* Cornu, *Erynia neoaphidis* Remaudière et Hennebert. Les deux *Entomophthoraceae* se sont montrées susceptibles de lysis sur le corp de l'insecte en peu de temps en permettent le suivant développement des saprophytes, lesquels masquent l'établissement des espèces parasites.

P. robinella

B. bassiana qui a été isolée des larves de ce lépidoptère, mineur des feuilles de *Robinia*, en épreuves d'infection en laboratoire, s'est révelée

très virulente à l'égard de l'insecte (95 et 100% de mortalité respectivement à 26 et à 15°C après dix jours). En épreuves préliminaires d'infection en champ, elle a provoqué dans les plantes qui l'ont reçue le 19% de mortalité des larves, en comparaison du 3% de celles témoins.

M. melolontha

Dix isolés de *B. brongniartii* (Sacc.) Petch ont été obtenus des larves du Hanneton commun (*M. melolontha*) provenantes des différentes localités de la région Vallée d'Aoste. Des valeurs de mortalité compris entre 70 et 100% dans des temps variables de 35 à 63 jours ont été atteints, selon les isolés, en essais d'infection sur larves de ver blanc agées du 2^{e} stade.

Des épreuves d'infection en champ sont programmées avec les isolés de *B. brongniartii* les plus virulentes.

BEAUVERIA BASSIANA SPORE PRODUCTION, VIRULENCE AND SURVIVAL

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Beauveria bassiana is being studied for the control of a range of agricultural pests including *Nephotettix* spp, *Ostrinia nubilalis* and *Hypothenemus hampei*. For commercialisation of *B. bassiana* it is vital to develop suitable production technologies and formulations that permit spore survival.

In a defined liquid medium *B. bassiana* produced conidia (max. $2.7X10^8$ spores per ml) while in a complex medium, hyphal bodies were formed (max. $1.6X10^9$ spores per ml). On semi-solid media up to $1.9X10^{10}$ conidia per gram were obtained. Spores produced in liquid culture were highly virulent to adult *Nephotettix virescens*. LC_{50} values were 1.5 and $2.6X10^6$ spores per ml for submerged conidia and hyphal bodies respectively, compared to 1.0 and $1.4X10^7$ for aerial conidia produced on Sabouraud dextrose agar or wheat.

Submerged conidia produced in liquid culture and aerial conidia produced on cereal grain and stored in water, survived similarly at 5°C and more than 60% were still viable after 180 days. Hyphal bodies survived less well and only 25% were viable after the same time. At 20°C, aerial conidia produced on cereal survived better than conidia produced in liquid culture. All the stored spore types maintained pathogenicity for at least 180 days.

When spore survival was examined under UV light (wavelength 253.7 nm), hyphal bodies and submerged conidia survived better than aerial conidia with LT50 of 4.6, 3.6, and 2.5 minutes respectively.

The implications of these results will be discussed.

SAFETY TO PARASITOIDS AND PREDATORS OF FUNGAL BIOCONTROL AGENTS

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There are over 700 described species of entomopathogenic fungi grouped in approximately 100 genera. Some fungi are presently used for the control of noxious insects in various parts of the world. Many more are under development and some are nearing receipt of governmental registration for field use.

A review of the present knowledge on potential fungal control agents indicates that these organisms pose a minimal risk to non-target invertebrates, including parasitoids and predatory insects. Indeed, when compared with chemical insecticides, fungal biocontrol agents offer, among other advantages, a method of control that has a very narrow host range, can usually be integrated with other biocontrol agents, and is also biodegradeable.

The best documented cases of detrimental effects of entomopathogenic fungi on non-target invertebrates are indirect effects on the predator and parasitoid populations through host depletion. In certain instances, fungi may also directly affect certain non-target invertebrates, including predators and parasitoids. It must be realized, however, that generally speaking, it is not possible to reduce the population level of a pest without also adversely affecting another component of the ecosystem. For instance, by reducing a host population, it is inevitable that the host's predators and parasites will be adversely affected. In such situations, it is the responsibility of pest managers to integrate the use of the biocontrol agents in order to utilize all control methods in the most efficient manner. For instance, the preservation of a beneficial insect may only require the proper timing of a fungal application.

In any case, the general consensus is that fungi do pose inherent albeit minimal risks and therefore should be regulated in some manner. Consequently, registration is of paramount importance. Most guidelines for registration of entomopathogenic fungi require laboratory testing for infectivity to non-target invertebrates. However, limitations of the present knowledge of fungal specificity and how it relates to epizootiology make it impossible to extrapolate such data to the field situation. Nevertheless, limited laboratory infectivity studies with the formulated product against non-target invertebrates may identify potential hazards that should be addressed during field trials.

With respect to the safety to invertebrates, safe and optimum use of fungi can only be determined for each specific integrated management system. To ensure maximum safety to invertebrates, fungal control agents must not become just a replacement to chemical insecticides, but should be integrated with other control strategies. This can only be accomplished if efficient formulated products are available to the researcher to test.

A major current limitation on the use of fungal biocontrol agents is the lack of formulated preparations. The private sector in turn is reluctant to develop a product which will then require extensive and costly safety testing prior to field evaluation. Therefore it is imperative that necessary safety regulations and registration procedures do not hinder the

development and consequent testing and utilization of such formulations. A better approach for ensuring long term safety to invertebrates would be continued monitoring during field use after preliminary laboratory and field safety testing.

This presentation reviews the relative safety to parasitoids and predatory insects of the presently registered as well as potential fungal control agents of insects and mites.