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GROUPE D'ETUDE "LES MICROORGANISMES ENTOMOPATHOGENES ET NEMATODES PARASITES D'INSECTES"

"MICROBIAL CONTROL OF WEEVILS AND ENVIRONMENTAL PERSISTENCE OF PATHOGENS AND NEMATODES"
"LUTTE BIOLOGIQUE CONTRE LES CURCULIONIDES ET PERSISTENCE DES GERMES ENTOMOPATHOGENES ET DES NEMATODES PARASITES D'INSECTES"

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INTERNATIONAL ORGANIZATION FOR BIOLOGICAL AND INTEGRATED CONTROL OF NOXIOUS ANIMALS AND PLANTS

WEST PALAEARCTIC REGIONAL SECTION

IOBC
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STUDY GROUP

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WORKSHOP / COLLOQUE, VERSAILLES (FRANCE)
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Introduction

The IOBC/WPRS study group on Insect Pathogens and Insect-parasitic nematodes was established in 1985. These proceedings arise from the first major meeting of the study group at Versailles in September 1987. This meeting took as its themes

a) The microbial control of weevils
b) Environmental persistence of insect pathogens

The present volume includes the abstracts of papers submitted at the meeting as well as summaries (rapporteurs' reports) of the many discussions during the meeting.

Concluding comments outline the potential areas of cooperation and collaboration between different members of the study group. A list of participants is included.

I would like to thank Dr.Jacques Fargues for the excellent manner in which he organised the meeting. My thanks are due also to Dr.P. Ferron (Head of the INRA Department of Zoology) and Dr.F. Rapilly (President of the INRA Centre of Versailles) for their generosity in allowing us to make use of the INRA facilities at Versailles.

Individuals wishing to participate in future meetings of the study group should contact the convenor.

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TABLE OF CONTENTS

CONTROL OF WEEVILS

(CURCULIONIDAE)

a) Use of Nematodes

IMPORTANCE OF BOTH VARIANTS IN *Xenorhabdus* spp., BACTERIA ASSOCIATED WITH ENTOMOPATHOGENIC NEMATODES, Steinernematidae AND Heterorhabditidae

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Non-feeding resistant infective stages of entomopathogenic nematodes carry bacteria in a special vesicle of their intestine (BIRD & AKHURST, 1983). During rearing of these animals, particularly on artificial medium, the feeding stages need these bacteria, which provide them with nutrients and transform their food (BEDDING, 1981). All Steinernematidae and Heterorhabditidae are associated with bacteria of the genus *Xenorhabdus* (POINAR, 1979), *X. luminescens* for Heterorhabditis spp., *X. nematophilus* (including 4 subspecies) for *Steinernema* spp.** (AKHURST, 1983). Monoxenic association between axenic nematodes and a culture of *Xenorhabdus* gives the best production of infective animals on artificial food and, after parasitism, in axenic insects (BOEMARE *et al*, 1983 a). Moreover, a variability was observed in *Xenorhabdus* spp. which influences the mass production of their host nematode (AKHURST, 1980; 1982). Native bacteria change in form in the course of *in vitro* culture and during the development of nematodes. The new form (secondary phase) is not as good as the primary phase in its ability to provide nematodes with nutrients (AKHURST, 1982).

Xenorhabdus spp. belong to Enterobacteriaceae (THOMAS & POINAR, 1979) although most of the strains lack some fundamental properties of this family. They are only distantly related to other genera of this family by DNA/DNA hybridisation (GRIMONT *et al*, 1984), but common antigen has been detected (RAMIA *et al*, 1982). The bacteria have crystalline and fibrous inclusions and in *X. luminescens* membranous structures (BOEMARE *et al*, 1983 b). The primary form adsorbs dyes (such as bromothymol blue on NBTA or neutral red on MacConkey agar), produces antimicrobial compounds, shows a lecithinase activity on egg yolk medium, is more heavily bioluminescent and shows differences in pigmentation when the native strain is bioluminescent and/or pigmented (BOEMARE & AKHURST, 1987). The primary forms contain crystalline inclusions which are much more difficult to detect in secondary form bacteria, where they are absent or very scarce. Moreover primary form bacteria in standard conditions are more-readily recognised (80%) by locust haemocytes (BREHELIN & BOEMARE, 1986) than secondary form (20%). A numerical taxonomic study of both phases of 21 strains of *Xenorhabdus* examined for 240 characters will be published shortly (AKHURST & BOEMARE, *in prep*).

The analyses point out the importance of recognising characters described above in examining the taxonomy of *Xenorhabdus* and also demonstrate a close correspondence between the taxonomic groups of *Xenorhabdus* and those of their nematode associates.

** (*Steinernema* spp. = *Neoaplectana* spp. + *S. kraussei* : WOUTS *et al*, 1982)

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ENTOMOPARASITIC NEMATODES IN THE CONTROL OF CURCULIONIDS

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Weevils attack many plant species damaging roots, bulbs, stems, leaves, fruits, etc.. Adults can usually be controlled by insecticides, but larvae in cryptic environments are often "problem-pests". In these latter cases, Steinernema spp. and Heterorhabditis spp. are logical tools for weevil control, due to their mobility and attraction to the target insect. Indeed, in pine stumps Dendroctonus frontalis (1) and Hylobius abietis (2) were parasitized to the extent of 40-60% and 66% respectively by Steinernema feltiae.

In Italy, in a two-year experiment, poplar trees were artificially infested with Cryptorynchus lapathi and treated with rhabditids (four replicates were used). Nematodes were introduced to the entrances of the weevil galleries (using cottonbuds) or sprayed on the trees. With the first method even S. feltiae caused 100% mortality, whilst with spraying, only S. bibionis gave acceptable control (75%) (3).

In the soil, successful trials were carried out against Curculio caryae (4), Hylobius abietis (5), and Nemocestes incomptus (6). Furthermore, against Otiorhynchus sulcatus, Heterorhabditis spp. are already commercially used in ornamentals in many countries.

In Italy, Heterorhabditis spp. gave good results in the control of O. sulcatus and O. salicicola on Cyclamen, Euonymus, Aralia, etc. (7). However, in order to obtain 90-100% efficacy, a high dosage of juvenile nematodes was used in contrast with the experience of many other authors. The dosage usually recommended is 100 infectives/cm^2 (= 6-8 inf./ml. soil). In Italy good results were achieved only by applying 5-6 times more nematodes. The necessity of high dosages is probably due to a number of factors, e.g.

a) There are different Otiorhynchus species in ornamentals beside the ones mentioned above, including O. armadillo, O. difficilis, O. aurifer, O. rugosostriatus.

b) There are nearly always later-instar larvae in the soil, and treatments cannot be timed accurately at the period of larval-hatching.

c) The low early-spring and late-autumn temperatures are limiting factors for the mobility of the Heterorhabditis species/strains used.

Experiments were carried out in Italy with two other weevil-species: Neoplinthus tigratus in glasshouses using S. glaseri [with variable results (8)] and with Cleonus punctiventris in a sugarbeet field. This latter trial was carried out in July 1987 using different species and strains of rhabditids. With each strain, 25-25 m^2 were irrigated with 10^6 nematodes/m^2. After two weeks, samples were collected from the plots. The assessment of the beetle larvae showed 70% parasitism by S. feltiae. However, whilst all larvae feeding on the roots were killed, those damaging the sugarbeet were rarely parasitized. The usual symptoms of Xenorhabdus infection were not observed in the beetle larvae. The juveniles apparently entered the mouth of the larvae and moved to the intestine, but could not enter the haemocoel. With the exception of 5% of 200 weevil larvae examined, nematodes were found only in

the intestine. From these juveniles, adults developed and these produced later only a small amount of new-generation juveniles as the space in the insect intestine was limited.

Notwithstanding the good results in the sugarbeet field, today's use of rhabditids on large areas such as sugarbeet fields is not yet economic.

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USE of HETERORHABDITIS SP. against OTIORHYNCHUS SULCATUS in SWITZERLAND

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The black vine weevil, Otiorhynchus sulcatus, is a very polyphagous insect attacking many ornamental plant species as well as grapevines and strawberries. The main centres of attack are nurseries, glasshouses and ornamental plants on root-gardens and in the surroundings of modern houses. The weevil may also penetrate these houses and become a nuisance to the occupants. As the use of toxic insecticides in these areas encountered increasing rejection in recent years, an alternative to chemical control is highly desirable. The observation in 1981 of Bedding and Miller and of Simons, showing Heterorhabditis spp. to be effective parasites of the larvae of O. sulcatus, opened a way for biological control. In Switzerland we began to rear Heterorhabditis in 1982. Among four nematode isolates obtained, we finally selected a Dutch isolate, Heterorhabditis sp., HW 79, which was reisolated after hibernation from an O. sulcatus larva. It was selected because of best yields in mass production. In 1983 and 1984 extensive control experiments were carried out in glasshouses and under field conditions. Summaries of the results are given in tables 1 and 2.

The positive results obtained in these experiments induced us to mass-rear Heterorhabditis sp. on artificial medium according to the method described by Bedding. Nematodes were first sold in 1985, and in 1986 mass production and commercialization were taken over by a cooperative society. Nearly 10,000 units, each containing 6 million nematodes, were sold in 1986; most of them were produced in our country, the remaining were imported. The application of nematodes is recommended to the potential buyer only, if certain preconditions are fulfilled:

a) Nematodes should be used only during that period of the life cycle of O. sulcatus when the larval stage is present.

b) The soil temperature should reach 12°C at least.

c) The soil moisture must be high during and a few days after application.

d) Efficacy is particularly high if nematodes are applied to potted plants, containers, borders etc., i.e. where the plant root system is somewhat restricted.

From a) and b) two application periods are feasible under the climatic conditions of our country; the first one from the end of April to the beginning of June, and the second one from the middle of September to mid-October. Under protected conditions (glasshouses etc.) these periods may differ.

As to dosages, the recommendation is up to 20,000 nematodes per litre of soil in pots and containers and 0.8 to 1 million per square metre for plants in soil.

Table 1
Efficacy of Heterorhabditis sp. against the larvae of O.sulcatus on potted plants in the glasshouse (Azalea, Euonymus,Strawberry,Grapevine)
Summary of results

Number of experiments	number of plants	surviving O.s.-larvae in controls	surviving O.s.-larvae in treatments	efficacy in %
7	168	639	0	100
2	74	289	3	99
1	24	123	13	89
1	8	30	4	85

Table 2
Efficacy of Heterorhabditis sp. against the larvae of O.sulcatus in practical field experiments
Summary of results

Locality	plants	% parasitism	observations
1	Vitis sp.(in pots)	73-82	surviving larvae in innermost part of root system
2	Euonymus fortunell	86-100	
3	Astilbe,Sedum,Saxifraga	85-100	
4	Sedum	60-85	many young,small larvae
5	Rhododendron,Erica	100	mostly last stage larvae

Lutte contre Otiorhynchus sulcatus en pépinière avec des nématodes du genre Neoaplectana (Steinernema)

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Des essais ont été réalisés avec les nématodes entomopathogènes Steinernematidae et Heterorhabditidae contre deux espèces de Curculionides: Otiorynchus sulcatus et Diaprepes abbreviatus:

a) Otiorhynchus sulcatus

Les essais ont été réalisés en septembre-octobre sur plants de pépinière (jeunes conifères) en collaboration avec le Service de la Protection des Végétaux Région Bourgogne. En 1985, les résultats sur les larves d'Otiorhynchus ont montré une efficacité de 100% avec Neoaplectana carpocapsae (souche francaise) multiplié sur insecte-hôte (Galleria mellonella) et 70% quand le nématode est produit sur milieu artificiel (méthode de Bedding améliorée). En 1986, l'efficacité a été de 96% avec Neoplectana glaseri et de 71% avec N. carpocapsae, les deux espèces étant produites sur Galleria.

b) Diaprepes abbreviatus

Un essai a été réalisé en mai-juin 1987 à la Martinique (Antilles francaises) contre les larves de ce ravageur qui attaquent les racines de Limettier (Citrus macrophylla) en pépinière et en verger. En collaboration avec l'INRA Antilles et le CIRAD (IRFA) Martinique, des plants de pépinière ont été traités avec N. carpocapsae et Heterorhabditis bacteriophora (souche argentine). Si N. carpocapsae s'est révélé peu adapté aux conditions tropicales, H. bacteriophora a montré une efficacité supérieure à 80%.

Ces essais montrent que l'on peut envisager très favorablement l'utilisation pratique des nématodes entomopathogènes contre les Curculionides, dans la mesure ou leur production pourra être assurée dans des conditions économiquement compétitives.

The use of rhabditid nematodes to control black vine weevil (Otiorhynchus sulcatus) in glasshouse ornamentals

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In the UK the most important pest of hardy ornamental stock and glasshouse ornamentals is Otiorhynchus sulcatus (F.). Its larvae feed on roots, rhizomes and corms and attack over 100 species of cultivated plants; adult weevils damage stems and leaves. In glasshouse trials at Littlehampton, a range of isolates of insect-parasitic rhabditid nematodes (Steinernema spp and Heterorhabditis heliothidis) have been used in trials with O. sulcatus-infested Cyclamen and Impatiens.

In one trial, 270 Impatiens (in 10cm square pots holding 0.6ℓ. compost) were each infested with seven O. sulcatus eggs. A day later, four batches of 54 plants were treated with a suspension of either Steinernema bibionis (isolate T319), S. feltiae (All), S. glaseri or S. kraussei; the remaining 54 plants were untreated controls. At monthly intervals for 12 weeks one-third of the plants were selected and their roots examined for O. sulcatus larvae (Table 1). Compost from the 18 untreated plants examined at week 12 was used in bioassays with Galleria mellonella larvae to determined whether or not nematodes had invaded from adjacent treated pots via the horticultural capillary matting upon which the plants had been standing.

Table 1 Total numbers of O. sulcatus larvae found in batches of 54 Impatiens plants

	No. weeks post-treatment		
	4	8	12
Untreated	36	39	22
S. bibionis	0	0	0
S. feltiae	3	0	2
S. glaseri	4	0	0
S. kraussei	0	0	0

Results (Table 1) showed that O. sulcatus was susceptible to all of the Steinernema spp. tested and that S. bibionis and S. kraussei totally eradicated weevil larvae. Bioassays confirmed that nematodes could migrate from plant to plant; 63% of Galleria were parasitised within 7 days of exposure to 3-month-old untreated compost.

In subsequent studies with the compost used in this trial, it was shown that when new Impatiens were grown in it, and when further inoculations of O. sulcatus eggs (7 per plant) were made, that nematodes were still active 8 months after they were originally applied. Again, S. bibionis and S. kraussei were the most effective parasites and afforded the greatest degree of protection to the plants (Table 2).

Table 2 Condition of weevil-infested Impatiens 4 months after planting into untreated compost or 4-month-old nematode-treated compost

	No. dead plants	% dead plants	Total no. weevil larvae
Untreated	37	69	83
S. bibionis	9	17	9
S. feltiae	26	48	50
S. glaseri	21	39	45
S. kraussei	10	19	18

In another trial the efficacy of S. bibionis (T319) and S. feltiae (All) was compared with that of two isolates of Heterorhabditis heliothidis (T327 and NZ). Cyclamen (192 plants in 10cm square pots) were infested with 15 O. sulcatus eggs and there were 48 replicates of five treatments (4 nematode isolates; untreated controls). The effect of nematode migration through capillary matting was assessed by placing half of the plants directly on the matting (to allow migration) and half in dishes on the matting (to prevent migration). The total numbers of live O. sulcatus larvae found in batches of 24 Cyclamen 4 weeks post-treatment are shown in Table 3.

Table 3 Numbers of O. sulcatus larvae found in untreated and nematode-treated Cyclamen

	Plants on matting	Plants in dishes
Untreated	195	225
S. feltiae	10	15
S. bibionis	9	1
H. heliothidis (T327)	5	0
H. heliothidis (NZ)	1	0

Nine parasitised weevil larvae were also found in untreated controls on matting; it is likely that the number of live O. sulcatus would have been further reduced if the plants had been examined six or eight weeks post-treatment instead of four weeks. The movement of nematodes between treated and untreated plants is interesting; it may have some significance in horticulture, particularly where isolated pest infestations suddenly develop some time after the grower has applied nematodes.

APPLICATION OF COMMERCIALLY PRODUCED NEMATODES AGAINST BLACK VINE WEEVIL

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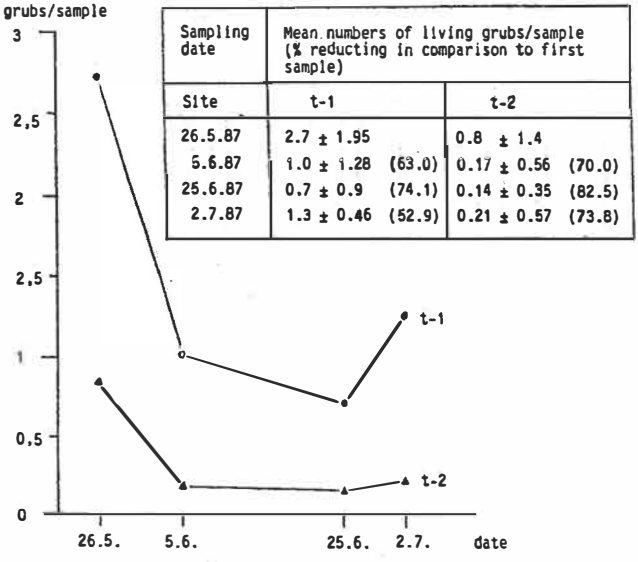
In spring 1987 entomogenous nematodes of the genus Heterorhabditis were applied in roof-gardens of the Max Planck Institut near Stuttgart to control black vine weevils. The infectives had been ordered from two Dutch firms, but all the nematodes were produced by only one of them. Counting the infectives short before application showed, that more nematodes had died during a 5 days shipping (31%) compared to 3 days (21%), respectively.

We applied $10^6/m^2$ infectives to the plant covering and washed down the nematodes with a water hose.

During may 1987 the soil temperature never exceeded 12°C, the threshold temperature for nematode activity. Counting the grubs in 1 l soil samples showed a decline of of the grub population (fig.). Because of the adverse weather conditions we found only a maximum reduction of 74-82% of the grubs in the roof-gardens. - As we were informed, there was a strong reduction of the weevils compared to the last year.

Therefore, a further application of nematodes is planned with additional spot treatments at places with high densities of grubs and/or very high and dense plant covering.



Sampling date	Mean numbers of living grubs/sample (% reducting in comparison to first sample)	
Site	t-1	t-2
26.5.87	2.7 ± 1.95	0.8 ± 1.4
5.6.87	1.0 ± 1.28 (63.0)	0.17 ± 0.56 (70.0)
25.6.87	0.7 ± 0.9 (74.1)	0.14 ± 0.35 (82.5)
2.7.87	1.3 ± 0.46 (52.9)	0.21 ± 0.57 (73.8)

fig. 2: Reduction of grubs per 1 l soil-sample after application of 1 x $10^6/m^2$ infectives of Heterorhabditis sp.
t-1: small terrace (about 300 m^2), t-2: large terrace (about 900 m^2).

Rapporteur's report

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Dr.EVANS chaired this first session, saying that the development of research on biological control of weevils is increasing in agriculture, horticulture and forestry because now some weevils are resistant to pesticides previously used for their control. The aim of this session was to introduce reports on field and glasshouse trials against weevils using entomopathogenic nematodes, particularly, against Otiorhynchus.

In the first paper Dr.BOEMARE said that attempts to rear nematodes are not yet entirely satisfactory due in part to the fact that the microflora of the nematode is often not well controlled. The purpose of his communication was to describe the variability of Xenorhabdus strains. All Steinernematidae and Heterorhabditidae are associated with a special genus of Enterobacteriaceae, Xenorhabdus spp. which provide nutrients for the growth of their hosts. All strains of Xenorhabdus are unstable and, after subculturing in vitro or in vivo (during nematode rearing), one phase, named the primary phase (which can be isolated from native infective animals), gives rise to a secondary phase. This secondary phase, which is a well defined bacteriological variant, is not so effective as the primary phase for providing infective juvenile stages during nematode rearing (in terms of larval number and physiological properties). Dr.BOEMARE presented several slides showing the phenotypic characters by which the two phases can be distinguished. He confirmed that this phase 1-phase 2 variation is common for all strains of the genus according to a numerical taxonomic study covering a worldwide sample.

During the discussion, because slides of protoplasmic crystalline inclusions of Xenorhabdus looked very much like the crystals of Bacillus thuringiensis in shape, Dr.PAYNE asked what their significance was (toxin or anything else?). These inclusions are smaller than B. thuringiensis crystals; each bacterium can contain several of them. Biochemical purification was made by GREGSON et al. (1987). These authors defined two types: one fibrous and the other crystalline, corresponding to two proteins of different molecular weight, but they did not find any insect toxin activity. Nobody yet knows their significance in terms of bacteriology and/or in respect of nematode physiology.

Another question was whether temperature was involved in the phase 1-phase 2 variation process. Dr.BOEMARE confirmed that temperature was important and that it is better to keep cultures at 14°c (not true for all the strains). However, this would not be the temperature used for long-term storage. The normal range of temperature for subculturing Xenorhabdus strains is 25-30°C. What is the reason for the phase 1-phase 2 change? Firstly, it is not known if it is a mutation. Recently, unpublished data of the BOEMARE team indicated that 3 specific proteins of phase 1 are obviously involved in the difference. When questioned about the defective phage described by POINAR (1980), Dr.BOEMARE replied that, because this phage was replicated on Bacillus cereus, it is difficult to decide if it comes from Xenorhabdus spp.

Dr.DESEO presented a report on the control of Curculionidae in Italy and compared her results with those published in other countries using rhabditid nematodes. First she pointed out the difficulties of taxonomy of weevils and listed the potential candidates as target insects for biological control by

nematodes: Otiorhynchus sulcatus; O. salicicola; O. armadillo; O. aurifer; O. rugosostriatus; O. difficilis; Neoplinthus tigratus; Cleonus punctiventris; Cryptorhynchus lapathi. Her trials showed that entomopathogenic nematodes, particularly Heterorhabditis spp., are very good for the control of weevils, particularly in the soil environment. On stumps of poplar, larvae of Cryptorhynchus lapathi were well controlled by Steinernema feltiae (97-100% mortality) if nematodes are introduced in insect galleries (2×10^4/cotton bud). By spraying (10^6/tree), S. bibionis was better than S. feltiae, giving 75% and 46-67% mortality, respectively. In sugarbeet, results were obtained with S. feltiae against Cleonus punctiventris (70% mortality with 10^6 nematodes/m^2). In this example the parasitism of Cleonus by juveniles exhibited a different pathology: they observed that the only point of entry was the mouthpart of the larvae. Nematodes invaded the intestine and they could not release their bacteria in the haemocoel. So larvae developed in the intestine and it was possible to find adults and, later, juveniles only in the intestine.

For the control of Otiorhynchus sulcatus on strawberries in the glasshouse they used H. heliothidis. With 35-50 nematodes/ml, 90-100% mortality was obtained (a dosage different from that of Bedding and Miller who used 10-40 nematodes/ml). During the discussion it appeared that SIAPA is developing the production of Heterorhabditis heliothidis for the control of these weevils where insecticides were not effective. At the present time this is only an experimental production using Galleria and a small number of nematodes produced in vitro using the Bedding method.

Distribution of nematodes is planned on sponge, as used by Biosis. For recovering nematodes after application to soil to examine their location and viability, Dr.DESEO and co-workers use a secondary host, the Galleria trap system.

Dr.KLINGLER pointed out also that chemical control was becoming impossible against O. sulcatus in the greenhouse. He followed Bedding and Miller in Tasmania and Simons in the Netherlands, in using Heterorhabditis. He produced a Dutch strain using the Bedding method, with a Swiss cooperative society. It is economic for high-value plants such as ornamentals with a dosage of 20,000 nematodes/litre of soil in containers and 0.8 to 1 million/m^2 for plants in the field. In the greenhouse, several treatments have to be made depending on the larval instar of the pest. In field conditions, two treatments are required in spring and autumn, depending on the life cycle of O. sulcatus and the temperature (12°c at least in soil). Dr.DESEO asked about the chance of persistence of nematodes in soil if a lot of target insects are killed. Dr.KLINGLER said that they did not find new juveniles after a defined period following application.

Dr.LAUMOND reported on trials of biological control of Otiorhynchus sulcatus with Neoaplectana carpocapsae (Plougastel strain). 100% mortality was obtained with nematodes reared on Galleria larvae and only 70% with nematodes reared with the Bedding method. Treatments were conducted on Chamaecyperis, an ornamental conifer, artificially contaminated with 40 eggs of O. sulcatus. Plants were treated with 2 million nematodes/m^2, on two occasions, separated by 15 days. Treatment of Taxus baccata, another species of conifer, showed a better success with N. glaseri (96% mortality) than with N. carpocapsae (70% mortality). These high dosages point out how essential it is to obtain a large mass production and, moreover, that trials below 12°C do not work. This year, trials were conducted in Martinique against Diaprepes abbreviatus (a root pest of Citrus macrophylla, the green lemon) in nurseries and orchards using N. carpocapsae and Heterorhabditis bacteriophora. The latter gave the best results (more than 80% control). However, the Plougastel strain was obviously unadapted to tropical conditions.

Dr.RICHARDSON reported recent trials in greenhouses using several rhabditid nematodes against O. sulcatus to protect ornamentals (Impatiens and Cyclamen). In the British Isles, 20 species of Otiorhynchus have been recorded, including 4 of economic importance: O. sulcatus, O. singularis, O. porcatus and O. aurifer. Four species of Steinernema, (S. bibionis, S. feltiae, S. glaseri, S. kraussei) and two strains of Heterorhabditis heliothidis (one from Tasmania, the other from New Zealand), were tested with three rates of treatment; low: 30,000 nematodes/pot; medium: 60,000 nematodes/pot; high: 120,000 nematodes/pot. Impatiens were cultivated in 10 cm^2/pots containing 0.61 litres of compost/pot and to each plant 7 eggs of O. sulcatus were applied.

Good control was obtained with the 2 species of Steinernema (S. bibionis, S. kraussei) and medium levels of control with two others (S. feltiae and S. glaseri). Three months after application, infective nematodes remaining in Impatiens rootballs were roughly proportional to the initial dosage. Eight months later, some viable nematodes can be detected in the compost, except for S. feltiae. A second experiment sought to compare two H. heliothidis strains versus S. bibionis and S. feltiae against O. sulcatus (15 eggs/plant) on Cyclamen, using 60,000 nematodes/plant. After 4 weeks the lowest number of surviving O. sulcatus was observed with the Heterorhabditis treatment. Thus, in the United Kingdom, Steinernematidae and Heterorhabditidae are practical agents for the biological control of O. sulcatus in glasshouse ornamentals. Infective nematodes persist well in compost and may migrate through horticultural capillary matting to colonize pest populations. Commercial use depends on their availability, cost-effectiveness, compatibility with chemicals and performance against O. sulcatus on other crops. In discussion, it emerged that Heterorhabditis is more effective over a short time period than Steinernema but is more difficult to rear. Treatments reported in U.K. glasshouses were often at 14-15°C, sometimes at 17-18°C and occasionally at 10°C.

Dr.BATHON had also used Heterorhabditis (10^6 nematodes/m^2) to control black vine weevil on Cotoneaster in roof gardens. In Germany, these insects were also considered highly insecticide resistant. Nematodes were bought from two Dutch firms. Applications induced a decline in soil populations but, unfortunately, adverse weather conditions (<12°C) did not permit more than 74-82% control. Additional applications are planned for next year. The discussion highlighted a problem of transport and the preservation of nematode activity. Heterorhabditis are more susceptible to postal delays than Steinernema.

Dr.EVANS chaired the final discussion. Some common factors emerged from the results presented by all the authors. Temperature is a limiting factor. Below 12°C the nematodes appear ineffective. Dr.VAN DER SCHAAF mentioned that he has some Dutch strains effective at lower temperatures. The goal is to select and to study new isolates of nematodes capable of growing and killing their target insects at lower temperatures. Moreover, bioassays must be conducted to define exactly the tolerance temperatures of nematodes. Another difficult aspect is to evaluate the movement of nematodes in soil; in sandy soil, for instance, nematodes circulated more easily.

CONTROL OF THE PEA WEEVIL, SITONA LINEATUS, BY MEANS OF NEMATODES

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The area of peas grown in the Netherlands has increased from ca. 4000 ha in 1982 to over 20.000 ha in 1986. Together with this increase in acreage also the problems with damage caused by pea weevils has increased. The damage caused by adults feeding on leaves is mostly cosmetical and generally not of major importance. Larvae feed on the root nodules and a 100% destruction of these nodules often occurs. This may lead to nitrogen shortage for the plants and thereby to lower yields. In particular in biological farming systems that depend on the culture of peas and beans for nitrogen fertilization the pea weevil causes problems. Biological control of the larvae with heterorhabditid nematodes was studied both in field and laboratory studies. A 100% parasitism was achieved in bioassays and small greenhouse plots using an equivalent of 1 million nematodes per square meter. The soil consisted of wetted sand. A field experiment in 1986 in peas on a loamy soil showed 45% larval mortality at a dose of 1 million nematodes per square meter. Dosages of 300.000 and 100.000 gave 34% and 14% parasitsism, respectively. These results show that there are in principle possibilities for this control method, but that at the moment the level of control in combination with the price of the nematodes (one guilder a million) makes practical application not feasible.

Prospects for the Biological Control of Hylobius abietis.

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The Large Pine Weevil, Hylobius abietis (L)(Col., Curculionidae) has been described as the most destructive insect pest in conifer reforestation areas.(Lindstrom A. et al.1986).

During the spring the eggs are laid in the stumps and roots of recently felled conifers. The larvae develop and feed on the cambium, without establishing a well defined gallery system.

Each summer both overwintering and newly emerged adults cause damage to seedling trees. Forestry Commission entomologists have estimated that there may be up to 750,000 adult weevils emerging from an average restocking hectare.

In 1987 approximately 17.5 million transplants were planted by the Forestry Commission. On average, 50% of unprotected transplants will be lost over the first two growing seasons. Therefore the cost of damage must be looked at in terms of the replacement of dead plants, planting, extra weeding and delayed revenue. This would lead to a loss totalling £1.8 million yr^{-1}. It has been predicted that the restocking programme will be doubled by the year 2000, while new planting areas will be reduced.

With this potential loss, considerable efforts have been devoted to protecting the transplants from weevil attack.

The Forestry Commission standard recommended technique to protect against weevil damage, is to dip plants in 1.6% Lindane(Gamma HCH) or 0.8% Permethrin. In 1984 1 metric tonne of the Lindane active ingredient was used. Research indicates that synthetic pyrethroids may be equally effective and new protocols for their use are being established. There are a number of arguments against the use of chemicals to protect plants. These include phytotoxicity from the chemicals themselves, increased plant handling during treatment increased planting costs due to the use of protective clothing and the worries about impact on non target organisims.

There are attractions in the possible development of economic forms of Biological control. Following the work of Pye and Pye (1985), the firt option in this area is the use of insect pathogenic nematodes.

However, there are a number of practical problems to be aware of, including, the means of application of the nematodes and the point in the weevil life cycle when the nematodes should be applied. There are two main options.

1. The larval stage. This is the most susceptible stage to invasion by entomogenous nematodes, but access to the larvae by the nematodes is difficult. It would be almost impossible to reach most of Hylobius abietis larvae developing in stumps and roots where, for example, larvae may develop in roots as small as 1cm in diam.

The position of pupation chambers under the bark varies with tree species. Access to the weevil larvae will be reduced in those trees with thicker bark.

It would be too labour intensive to inject nematodes under the bark while nematodes sprayed onto the stump are likely to dry out. If introduced to the soil around the stump, nematodes will be dependant on a strong attraction to the weevil larvae and would require high soil temperature together with need for considerable mobility.

Treatment of the larvae would require either the application of nematodes in late August, when the stumps are fresh and the H abietis larvae are developing to the final Instar, or in May, when the stumps are decaying, larvae are in the pupation chambers, and the soil temperature is relatively cold for nematode movement.

2. The adult stage. Access by the nematodes would be improved if applied to adult weevils. This is feasible because adult H abietis burrrow into the soil around the transplant roots and remain there for long periods of time. Nematodes applied to the roots would, therefore, be provided with a protected environment and would be available to attack the damaging adult population and reduce immediate damage.

Although the adults are not as susceptible as the larvae to invasion, environmental conditions may be improved to facilitate nematode movement and invasion. For example:

(a) Transplants are increasingly planted on mounds of soil to increase the soil temperature and therefore improve the rate of root development. This also results in more favourable conditions for nematode movement.

(b) Nematode suspension may be added to the root system of transplants in 'Japanese Paper Pots' before planting to increase the protection of nematodes against dessication.

(c) Nematodes may be sprayed around the roots of bare rooted transplants using conventional spraying equipment.

However, before any decisions can be made, research must continue to determine the correct nematode species and strain that will control H abietis under its varied environmental conditions, in combination with a more detailed knowledge of H.abietis behavioral patterns.

Pye A.E. Pye N.L. (1985). Different applications of the insect parasitic nematode Neoaplectana carpocapsae, to control the Large Pine Weevil, Hylobius abietis.Nematologica 31: 109-116.
Lindstrom A. et al. (1986). Field performance of a protective collar against damage by Hylobius abietis. Scand.J.For.Res. 1:3-15.

Rapporteur's report

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The first paper by P.H.Smits dealt with a study on the control of the pea weevil, Sitona lineatus on peas with nematodes of the genus Heterorhabditis. In laboratory and greenhouse experiments 100% larval control was achieved with a dose of one million nematodes per m^2. Under field conditions in peas on a loamy soil only 45% control was achieved. The price of the nematodes together with the relatively high dose required makes the practical use of this control method unlikely in the near future.

S.Collins and H.Evans reported on the control of the large pine weevil, Hylobius abietis, by means of Neoaplectana (=Steinernema) carpocapsae. Large pine weevil is a problem in areas that are replanted with young trees. Estimated damage is over 1 million pounds a year. At the moment, Lindane is used for control. Nematode applications have reduced the number of killed trees from 50-60% to only 11%. A major problem is the application. Bark and soil condition show great variability. Applications in spring give problems as temperatures are too low for nematode activity. Suggestions are to apply nematodes against overwintering adults or to apply them to trees before they are planted.

Further discussion of these papers was included in the following round table discussion.

ROUND TABLE DISCUSSION; The use of nematodes for control of weevils

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Discussion was structured around a number of key topics that were thought to be important in defining the pathogenicity and survival of nematodes in control programmes.

1. Temperature tolerance.

Discussion centered on work, carried out by Dr Deseo, which indicated that, within single species of nematodes, different strains have differing temperature thresholds. For example at temperatures of 18C there were marked differences in the responses of several nematode species such that, of 18 strains of *Heterorhabditis heliothidis*, 7 had no activity, 11 induced mortality in the test insect and none multiplied. By contrast, 21 strains of *Steinernema feltiae* had responses of 9 showing no activity, 4 killing the host and the remaining 8 multiplying in the host. Thus, even at 18C, there was a lowering in both efficacy and multiplication.

The problem was therefore seen as finding more cold tolerant strains of nematode for field use. Bioassays have been used to assess cold tolerance and one suggestion, based on Deseo's work, was to measure migration on paper strips which gave a qualitative measure of temperature tolerance. However, this only worked for *Steinernema* species. It was also thought that temperature affected not only activity but also the ability to penetrate the host and this aspect should also be considered in searching for new strains of nematode. Dr Griffin described work in Ireland that indicated that the Dutch strain of *Steinernema* was able to tolerate 12C and migrated at these temperatures. Dr Bathon provided support for this view in describing some of the work being carried out at Darmstadt while similar results were reported from Dr Simons laboratory in the Netherlands.

Discussion then centered on the scope for isolating new temperature tolerant strains from different parts of the world. Dr Griffin described the isolation of a

Heterorhabditis strain from Ireland but this had not been characterised. Selective breeding of these new strains was thought to be a possibility. Paul Richardson described the isolation of some cold tolerant strains (10C) from Sweden and Newfoundland but felt that mobility and bacterial multiplication could be a problem.
More detailed discussion on specific examples of the links between temperature tolerance and genetic variability then followed. Dr Klingler described the 12C threshold for activity displayed by Australian strains of nematode while caution was expressed concerning the applicability of laboratory results to the field situation.
The perceived requirements in respect of temperature tolerance were :
(a) Improved bioassay methods for testing temperature effects.
(b) Screening for temperature tolerance using these improved bioassays.

2. Integration of nematodes and other control agents.

The discussion was opened by asking for any experiences of use of nematodes with other agents or of examples of effects from inadvertent use of chemicals.
Experience in the Netherlands indicated that insecticides did not seem to affect nematodes, while one month after use of nematicides there was no impact on applied nematodes. In Italy there was a list of agents that had been tested for effects and only benomyl was known to have any impact on nematode survival. Paul Richardson stated that, in the control of mushroom flies, chemicals such as diazonin and dimilin were synergistic when used at the same time as nematodes. Dr Kenneth confirmed the problems associated with the use of fungicides and pointed out that TBZ was lethal to nematodes. Dr Zimmerman described experiments to use *Metarhizium* with nematodes which indicated that they could be used together but that they were not synergistic. He also

stated that more needed to be known about the relative persistence of both agents.
The conclusions on this aspect were that we need :
(a) Information on persistence of both nematodes and other agents.
(b) More data on joint actions with other agents, particularly pathogens.

3. Impact on non-target organisms.

Discussion on this aspect was brief since little work has been carried out systematically to test for the impact of nematodes on non-target organisms, either beneficials or other pests. Dr Smits described some experiments on control of *Sitona lineatus* in plots treated with the Dutch strain *HL81*. He reported that total numbers of larvae in treated plots were higher in the following years and thought this may bave been due to loss of predators, although he had no direct evidence. A call was made for plans to carry out studies in this area of research.
To this end a much better set of experiments on host ranges of nematodes was needed.

4. Characterisation and identification.

The topic was introduced by asking whether we had the methods or the reproducibility to accurately characterise the species and strains of nematodes available for use in control programmes. The view from the Netherlands was that taxonomy was in a mess and that researchers were not even using the correct specific names. The question of using bacteria as a taxonomic tool was discussed but in general was dismissed since the same bacteria may be present in at least three different groups of nematodes. Similarly it was not possible to use the host insects as criteria.
Dr Laumond pointed out the 1984 paper by Poinar describing the determination of *Steinernema* into four groups. It followed that field experiments required an accurate characterisation both to species and to strain.

Biochemical characterisation had been attempted with some success and it was clear that DNA technology was applicable to nematodes and that some work had been carried out in the USA. However, the technology involved was likely to stay out of the reach of the majority of researchers involved in the practical use of nematodes. Paul Richardson pointed out that the adult is the easiest stage for the trained taxonomist while Dr Godliman pointed out that DNA fingerprinting could be important if different strains of the same species were used.
Dr Deseo also referred to data sheets for characterisation of species produced by Ackhurst & Bedding and made a plea that these sheets should be made more widely available.
The research needs on this aspect were thought to be:
(a) Provision of a central collection of nematode species and strains.
(b) Development and distribution of a standardised method for characterisation of nematode species and strains.

5. Production and patenting of nematodes.

Industrial production of *Heterorhabditis sp.* was thought to the main problem facing commercial expoitation of nematodes. Dr Schaaf agreed that problems existed and that they may revolve around the bacteria associated with the nematodes. Dr Boemare agreed and also thought that media are important especially the nutrient balance. For field use it was thought that entry of the L3 stage into the host in the field was important.
On the question of patenting, it was clear that difficulties arose when some countries were covered and others not. An example was Bedding's "bag" method which was patented in several countries but not the Netherlands and the UK. Dr Laumond pointed out that he thought Bedding's method was not suitable for large scale industrial use and that it would be better to look at fermentation methods in order to exploit existing equipment. Dr Klingler used Bedding's method and thought that bacteria in the primary phase were very important.

This final session on research needs identified the following areas for further study:

(a) An appraisal of the relative costs of the various rearing methods was needed.

(b) Was there any effect of adaptation to different hosts and did this influence pathogenicity or host range in the field?

(c) How far can scientists exchange information freely without compromising commercial exploitation of their results?

Paul Richardson was then asked to compile a list of possible research topics for discussion among the nematologists on the final day of the meeting.

The session ended with a general discussion on the problems of using nematodes against weevils.

Dr Klingler was concerned about the taxonomy of the target insect hosts themselves which was reiterated by Paul Richardson who pointed out that it was necessary to grow the larval stages through to adult to be sure about the identification. Dr Smits asked if anything was known about carbon dioxide gradients and was referred to the proceedings of the SIP meeting at Veldhoven.

This was a valuable discussion session that highlighted a number of potential research topics that were more fully discussed at the final round table session on 4 September.

CONTROL OF WEEVILS

(CURCULIONIDAE)

b) Use of Fungi

Entomophthorales on Coleoptera: a collation of host/pathogen associations

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In the fungal order Entomophthorales (Zygomycetes), comprising 4 or more families, species of most, but not all genera are known to attack insects and mites, and all species of some genera appear to be obligately parasitic upon them, e.g. Neozygites, Massospora. In certain other genera, particularly Conidiobolus, some species are apparently truly saprophytic, whereas others on occasion may attack insects. Only a few years ago the large, unwieldy and heterogenous genus Entomophthora was revised, with some species being incorporated into Conidiobolus and others being placed in newly erected genera, leaving Entomophthora sensu stricto depauperate. The basis for these changes was mostly on cytological grounds, but this wise step in taxonomy is now allowing biological differences to begin to surface, such as obligate parasitism in all Neozygites species, the ability of Conidiobolus and Erynia species to grow in mass culture and to possibly survive saprophytically in nature, and host range proclivities, etc.

By far the greatest number of both host and pathogen genera and species connections are known from Diptera and Homoptera, but many other insect orders contain host species. The order Coleoptera is not as well represented as the above two, in spite of its size. Furthermore, if we compare the genera of beetles infectible by hyphomycetous fungi such as Beauveria spp., Metarhizium and Paecilomyces spp., or by species of Cordyceps or by the Laboulbeniales, with those reported as host of entomophthorous fungi, the former are much more strongly represented, and indeed, most of the

literature is about them. Nevertheless, a perusal of the literature leads us to realize that: (1) Entomophthorales-beetle pathogenic associations are actually commoner than one would initially suspect. (2) Some of the infections eventually reach epizootic proportions. We badly need a complete and annotated collation of all these connections on Coleoptera, much like that published by Mlle Genevie'ye Thoizon in 1970 "Specificite du parasitisme des Aphides par les Entomophthorales". Macleod and Muller-Kogler (1970,1973) had partial lists in which Coleoptera were included. Here, we give only the "bare bones", so to speak, of a global collation. It needs more clarification, but hopefully it will spur others to provide further information and data, so that we can fill in gaps and perhaps be able even to predict to a large extent the host limits of some genera, etc. For instance: which superfamilies, families and genera are particularly prone to such infection? What is the place of geography, of climate, of season? Which stage(s) is infectible and what are the possible reasons for stage infectibility or the converse, - could it be a particular habitat during a life stage, such as foliage, soil, within plant tissues or on plant detritus? Or does the physical structure of particular stage confer immunity, for, as an example, there are 6 different kinds of larvae among the beetles? Unfortunately, the literature abounds with articles in which the susceptible stage(s) cannot be guessed (and the senior author himself pleads guilty in one instance). When a particular stage of a beetle genus proves infectible to a hyphomycete and this same genus comprises one or more host species of an entomophthorous fungus, will it be the same stage that is affected by the latter, or another one, and why?

Species in the following entomophthorous genera are recorded as attacking Coleoptera: Erynia, Conidiobolus, Eryniopsis, Entomophthora, Tarichium and possibly Entomophaga. So far, the following genera of insect-attacking Entomopbthorales have not been reliably reported on Coleoptera: Neozygites, Strongwellsea, Massospora, Thaxterosporium, Meristacrum.

Conidiobolus spp. on beetles are undoubtedly sometimes opportunistic, but we hold that C. osmodes in Israel could be a primary pathogen of

Hypera variabilis in the field, and C. major (=C. apiculatus var. major) was often found to be common on adult soldier beetles in USA and C. apiculatus on adults of Trochalis and Adoretus in South Africa. In Tarichium species, only the resting spores are known, making identification uncertain, unless a conidial phase is eventually discovered in the same individuals, at which time the species should no longer be considered a Tarichium. By far the most heavily represented genus is Erynia, and of its 4 subgenera, both Zoophthora (in which special secondary spores, the capilliconidia, are produced) and Neopandora (in which they do not form) have many such species. Entomophthora sensu stricto has only lately been found on a beetle, in Denmark, and only one species of the newly erected genus Eryniopsis is on a beetle.

TABLE 1

Genera and Species of Entomophthorales attacking Coleoptera

Fungal Genus & Species	Host Family	Host Genus & Species	Susceptible stage	Fungal stage conidia=Con resting spores=RS	
Eryniopsis Humber					
E. lampyridarum	Cantharidae	*Chauliognathus pennsylvanicus* (soldier beetle)	Adult	Con	
Conidiobolus Brefeld					
C. osmodes	Curculionidae	*Hypera variabilis*	Larva, Pupa	Con	RS
		Hypera postica	Larva		
C. thromboides	Tenebrionidae	*Tribolium destructor*	Larva	Con	RS
C. major (=*C. apiculatus* var. *major*)	Ptilodactylidae	*Ptilodactyla serricola*	Adult	Con	?
C. apiculatus	Scarabeidae	*Trochalis fulgidus*	Adult	Con	
	Scarabeidae	*Adoretus ictericus*	Adult	Con	
C. coronatus	Curculionidae	*Ceutorhynchus napi*	?		
Entomophthora Fres.					
Entomophthora sp. nr. *muscae*	Cantharidae	*Cantharis*	Adult	Con	?
?*Entomophaga* Batko					
? *E. grylli*	Cerambycidae	*Saperda carcharias* (timber beetle)	?	?	?
	Silphidae	*Aclypea undata*	Larva	Con	RS
Tarichium Cohn sensu Lakon					
T. cleoni	Curculionidae	*Cleonus (Bathynoderes) punctiventris*	Larva, Pupa		RS
? *T. coleopterorum*	Curculionidae	*Sitona flavescens*	Larva, Adult	?	RS
	Elateridae	*Elater* sp.			RS
	Curculionidae	*Sitona sulcifrons*	Larva		

	Curculionidae	Sitona lepidus	Larva		
	Curculionidae	Sitona hispidulus	Larva		
? I. carpentieri	Elateridae	Agriotes sputator	Adult		RS
(probably is	Elateridae	Elater sp.	?Adult		RS
Erynia anglica					
(=E. elateridiphaga)					
I. rhagonycharum	Cantharidae	Rhagonycha lignosa	Adult		RS
Erynia Batko					
Subgenus Zoophthora					
E. phytonomi	Curculionidae	Hypera (Phytonomus)			
	(Subfam.	punctata			
	Hyiobriinae)	(=H. variabilis)	Larva	Con	RS
		Hypera postica	Larva	Con	RS
E. anglica	Elateridae	Agriotes sputator	Adult	Con	RS
(=E. elateridiphaga)		Agriotes obscurus	Adult	Con	RS
? E. anglica, acc.to Petch	Chrysomelidae				
	(subfam. Galerucinae)	Galerucella			
(mixture of fungi & hosts)		tenella	Larva	?	?
E. crassitunicata	Cantharidae	(?)Malthodes sp.	?	Con	RS
Subgenus Neopandora					
E. brahminae	Scarabeidae				
	(subfam. Rutelinae)	Brahmina sp.	Adult	Con	RS
	do	Anomala			
		rufiventris	Adult	Con	RS
E. zabrii	Carabidae				
	(subfam. Pterostichinae)	Zabrus			
		tenebrioides	Larva	Con	RS
E. suturalis	Chrysomelidae	Lochmaea suturalis	Adult	Con	RS
Erynia sp. (undescr.)	Carabidae				
	(subfam. Trechinae:				
		Trechus quadristriatus	Adult	Con	
Erynia sensu lato					
E. nebriae	Carabidae				
	(subfam. Nebriinae)	Nebria			
		brevicollis	Adult	Con	?
Erynia sp. (undescr.)	Cantharidae	Rhagonycha lignosa	Adult	Con	RS
Erynia sp. (undescr.)	Curculionidae	Hypera postica	Larva	Con	RS
Erynia sp. (undescr.)	Silphidae	Silpha undata			
		(=Aclypea undata)	?		

Looked at in a different manner, (Table 2), the most commonly stricken coleopterous group appears to be the Curculionidae (weevils), with many species attacked, by subgenus Zoophthora of Erynia, by Conidiobolus and by the problematical (for identification) Tarichium. Cantharidae and Carabidae are also common hosts, with Erynia (subgen. Zoophthora), Eryniopsis and Entomophthora on the former and Erynia (subgen. Neopandora) on the second. All 3 families have also taxonomically unresolved species of Erynia attacking them. Furthermore, some records might be in error or in contention e.g. a mixture of fungi and hosts in T. Petch's collection of Galerucella tenella, supposedly with E. anglica, or some species of Tarichium on beetles.

TABLE 2

Coleoptera attacked by Entomophthorales, arranged according to insect families

SUB-ORDER ADEPHAGA		
CARABIDAE		
Erynia (subgen. Neopandora) zabrii	Zabrus tenebrioides	Larva
Erynia (sensu lato) nebriae	Nebria brevicollis	Adult
Erynia sp. (subgen. Neopandora)	Trechus quadristriatus	Adult
SUB-ORDER POLYPHAGA: HAPLOSCLETA		
SCARABEIDAE		
(subfam. Rutelinae)		
Erynia (subgen. Neopandora) brahminae	Brahmina sp.	Adult
	Anomala rufiventris	Adult
Conidiobolus apiculatus	Trochalus fuligius	Adult
	Adoretus ictericus	Adult
SILPHIDAE		
Erynia sp.	Silpha (=Aclypea)	
	undata	
STAPHYLINIDAE		
Erynia (subgen. Neopandora) ?suturalis	Beetle	Adult
SUBORDER POLYPHAGA: SYMPHIDOGASTRA		
PTILODACTYLIDAE		
Conidiobolus major	Ptilodactyla serricola	Adult

ELATERIDAE		
Erynia (subgen. Zoophthora) anglica (=E. elateridiphaga)	Agriotes spp.	Adult
? Tarichium carpentieri (probably E. anglica)	Agriotes sputator	Adult
	Elater sp.	Adult
? Tarichium coleopterorum	Elater sp.	?
CANTHARIDAE		
Entomophthora sp. (nr. muscae)	Cantharis	Adult
Eryniopsis lampyridarum	Chauliognathus pennsylvanicus	Adult
Erynia (subgen. Zoophthora) crassituunicata	(?)Malthodes sp.	?
? Erynia (subgen. Zoophthora) anglica	Cantharis	Adult
Erynia sp. nov.	Rhagonycha lignosa	Adult
Tarichium rhagonycharum	Rhagonycha lignosa	Adult
TENEBRIONIDAE		
Conidiobolus thromboides (as Entomophthora virulenta)	Tribolium destructor	Larva
CERAMBYCIDAE (=Timber beetles)		
? Entomophaga oryili	Saperda carcharias	?
CHRYSOMELIDAE (=Leaf beetles)		
Erynia (subgen. Neozygandora) suturalis (subfam. Clytrinae)	Lochmaea suturalis	Adult

? Erynia (subgen. Zoophthora) anglica	Galerucella tenella	Larva
CURCULIONIDAE		
Erynia (subgen. Zoophthora) phytonomi	Hypera spp.	Larva
Erynia sp. sensu lato	Hypera postica	Larva
Tarichium cleoni	Cleonus (Bathynoderes) punctiventris	Larva, Pupa
?Tarichium coleopterorum	Sitona spp.	Larva, ?Adult
Conidiobolus osmodes	Hypera	Larva, Pupa
Conidiobolus coronatus	Ceutorhynchus napi	?

From the above Tables, we can sum up a few points: (1) At least 5, perhaps 6 genera of Entomophthorales are now known to attack Coleoptera. (2) At least 11 families of Coleoptera have host species. (3) The host families range over almost the entire phylogenetic spectrum of the Order. (4) At least 3 entomophthorous genera may be found on each of at least 2 beetle families e.g. Erynia, Conidiobolus and Tarichium on Curculionidae, and Erynia, Eryniopsis and Entomophthora on Cantharidae. (5) Some fungal genera and even subgenera may be found on a number of beetle families e.g. Erynia subgen. Zoophthora on Curculionidae, Elateridae, Cantharidae, and perhaps Chrysomelidae; Erynia (subgen. Neopandora) on Scarabaeidae, Carabidae, Staphylinidae and Chrysomelidae; Conidiobolus on Curculionidae, Tenebrionidae and Ptilodactylidae. (6) Entomophthorales on Curculionidae are perhaps primarily, if not completely on larvae, whereas on Elateridae and Cantharidae they appear to be mostly if not completely restricted to adults. If so, why? (7) Stage(s) of stricken insects should always be given in the literature in the future.

Endémisme des mycoses à Beauveria bassiana et Metarhizium anisopliae dans des populations naturelles d'Otiorhynchus sulcatus

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L'étude de l'état sanitaire de populations naturelles d'Otiorhynchus sulcatus (Plougastel-Daoulas, France) a permis de mettre en évidence la diversité de la flore cryptogamique entomopathogène qui affecte les phases souterraine et aérienne du cycle de ce ravageur: Beauveria brongniartii, B. bassiana, Metarhizium anisopliae, M. flavoviride, Paecilomyces fumosoroseus, P. farinosus, Gliocladium sp. Deux germes sont fortement représentés sur l'ensemble des sites du biotope étudié:

Beauveria bassiana, dont la présence dans le sol des fraiseraies ne déclenche qu'une faible mortalité au stade larvaire, avec un taux d'endémisme de 0.6% pour un effectif n = 3377 individus élevés en quarantaine, mais détermine une mortalité cryptogamique différée au stade adulte variant de 6 à 60% selon les sites étudiés.

Metarhizium anisopliae est présent dans toutes les populations larvaires des sites du biotope. Si dans la majorité des cas, la mortalité est inférieure à 10%, elle peut atteindre des taux plus importants, 15, 20, 50 et 70%.

A l'inverse, les populations adultes d'O. sulcatus plus sujettes à l'infection due à B. bassiana, ne subissent pas ou peu la pression de M. anisopliae, les taux de mortalité sont toujours inférieurs à 10%.

Pour ces deux Hyphomycètes dominants la contamination s'effectue durant le stade larvaire de l'hôte; le processus infectieux évolue pendant ce stade pour M. anisopliae alors que B. bassiana semble plus inféodé à la phase aérienne du cycle d'O. sulcatus.

Les diverses situations épidémiologiques observées pourraient être liées, pour partie, à des différences de niveaux des populations de chacun des deux microorganismes ainsi qu'à des phénomènes d'antagonisme ou de compétition au niveau même de l'insecte-hôte.

THE USE OF FUNGI TO CONTROL THE BLACK VINE WEEVIL, *OTIORHYNCHUS SULCATUS* ON ORNAMENTALS

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The black vine weevil, *Otiorhynchus sulcatus*, is a serious pest of ornamentals both on glasshouse and outdoor crops. Aldrin provides effective control but its use has been restricted due to concern over this compound's prolonged environmental persistence. There is a clear need for alternative methods of vine weevil control and both fungi and nematodes have shown promise. Of the fungi, several isolates of *Metarhizium anisopliae* have provided good control of vine weevil larvae on both ornamentals and strawberries under glass. This paper describes the screening of fungi for weevil control on glasshouse-grown Begonia and reports the results of some laboratory assays comparing virulence of three *M. anisopliae* strains.

Of twenty one fungal isolates from the genera *Beauveria* (6 strains), *Metarhizium* (13) and *Paecilomyces* (2) examined, three strains of *M. anisopliae* reduced larval populations on Begonia plants by over 85%, while one (*M. anisopliae* 35-79) provided complete control. These results are promising as the dose used (10^{13} spores per ha) could be produced on 2 kg of barley grain in about 10 days.

In the laboratory, application of conidial suspensions (10^{4}-10^{7} conidial ml^{-1}) of *M. anisopliae* strains 35-79; or 275-86 and 276-86, kindly provided by Zimmermann, to weevil-infested Begonia plants gave similar levels of control, irrespective of strain. There was a weak dose mortality response (mean slope of regression line 0.6) and LC_{50} values after 21 days were approximately 10^{6} conidia per ml.

Vine weevil larvae of various instars survived well in the laboratory when maintained in peat-filled Petri plates and fed on Impatiens roots. Mortalities of first, third, or fifth instar larvae maintained in Petri plates previously treated with conidial suspensions of *M. anisopliae* 35-79, 275-86 or 276-86, were similar and again showed a weak dose mortality response; the data showed the fungi were most active against early instar larvae.

It is considered fungi have potential for controlling *O. sulcatus* on glasshouse ornamentals and could be developed as an economic method for control of this increasingly serious pest.

BEAUVERIA BRONGNIARTII AS A BIOLOGICAL CONTROL AGENT AGAINST OTIORHYNCHUS SULCATUS

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In Belgium, as elsewhere, the damage due to Otiorhynchus sulcatus appears to increase with improvements in cultural practice. The pest's rise seems mainly due, during these ten last years, to the use of containers in horticulture and nursery production. In these conditions, the soil, the microclimate and the avoidance of natural enemies seem to promote the weevil's multiplication.

The insect is parthenogenetic and quite prolific, laying about 1,600 eggs per year giving rise to about 400 adults. The pest's annual cycle presents large variations influenced by variations in climate. In the laboratory, we have observed adults laying eggs for more than two years. Both larva and adult are largely polyphagous on more than 250 plants but the root-feeding larva is the most damaging stage, often leading to plant death.

Effective chemical insecticides are few and are chiefly active against adults; the infrequent use induces a rapid development of resistance. P. FERRON (1978), in France, and S. KELLER (1986), in Switzerland, obtained good results with the fungus Beauveria brongniartii against Melolontha larval instars.

Our first tests were performed "in vitro" and, with one exception, we used the last larval instar. For each test 10 larvae are placed per Pétri dish containing 18g compost and 2g Norway spruce (Picea exelsa) roots. Three replicates are used. Beauveria spore suspensions are made at 10^7 and 10^8 conidia per ml in distilled water with 5% Tween 80. The conidia are collected from a 14 day-old culture at 25°C. 2 ml conidia suspension are poured per dish. The check consisted of 10 larvae (3 replicates) treated with distilled water with 5% Tween 80. First we tested 1 strain of Beauveria bassiana and 3 strains of Beauveria brongniartii. The two B. brongniartii strains seemed to be the most virulent, killing all the larvae between 14 to 21 days at 25° or 18°C.

With these strains, death of 83-97% of larvae occurred even at 15°C within 28 days, using 10^8 conidia per ml.

One test performed on small larvae seems to indicate that these are more sensitive to infection.

Our second experiment was performed "in vivo" in the greenhouse with the most virulent strain of B. brongniartii, using 10^8 conidia/ml at the rate of 10 ml per 6 litre pot containing poplars or azaleas and infected with 10 or 12 larvae (third instar) or 600 eggs. Direct treatments, i.e. treatments 7 or 12 hours after larval introduction killed up to 84% of the larvae. Preventative treatment, 5 weeks before larval introduction, killed an average of 57% of the larvae. Delayed treatments killed 72 to 76% of the larvae.

The results were consistent regardless of the plant species used. One test, on azaleas, gave less satisfactory results; this seems to be due to high temperatures (52°C) reached in the greenhouse just after treatment. Nevertheless, the fungus was not dead, we could isolate it again from the compost at the end of the test.

If we compare our two experiments; it is clear that it is impossible to monitor the death of the larvae, in the "in vivo" tests, by detailed examination of the pots each 3 or 7 days. We had to utilize physiological techniques to measure the health status of the plants (see the next paper).

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NON-DESTRUCTIVE METHODS FOR ESTIMATING ROOT DAMAGE DONE TO EXPERIMENTAL PLANTS

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INTRODUCTION

The recording of larvae of Otiorhynchus sulcatus infected with insect-pathogenic fungi can be achieved daily in vitro, but when the plants are in soil they can only be examined once at the end of the experiment. We followed the efficiency of control treatments for O. sulcatus by observing stomatal conductance, foliar potential and free proline accumulation.

Root damage affects water uptake. In stressed plants, gas exchange is limited. Using Fick's assumptions of gas diffusion, we may explain water flow within plants as an electrical system composed of resistances and generators.

PHYSIOLOGICAL MODEL

Two hydric potentials are determined by environmental factors: in soil (water contents and cohesion forces) in air (wind speed and relative humidity). In plants, water flow has to pass through the Casparian band of the endodermis, viscosity within the vascular system and diffusion through the cuticle. Ionic pumps (absorbent hairs) and photosynthesis create an indigenous potential. (fig 1)

According to Edwin's formula:

$$\Delta\Psi = -J \cdot \left(\frac{R_f}{\Omega_f} + \frac{R_r}{\Omega_r} + \frac{8\rho\Delta X}{\Sigma r^4}\right) + \frac{\Omega_r R T J_s^*}{J}$$

and

$$J_s^* = fonction\ (\zeta_{ions}, \Omega_{absorbing}, \ldots)$$

with :

air-soil potential drop	$\Delta\Psi$	ionic salt composition	ζ_{ions}
water flow	J	solute flow	J_s^*
global root resistance	R_r	roots index	Ω_r
global foliar resistance	R_f	foliair index	Ω_f
lenght of xylem elements	ΔX	radius of xylem element	r

These assumptions include the root index and can be used to forecast the turgor of plants affected by different treatments against root pests.

Increasing water retention forces in the soil or the destruction of water absorbent surfaces will cause an increase in foliar resistance. If stress continues for longer periods, other physiological modifications occur e.g. changing the lipid composition of membranes, increasing free proline etc. If we use plant clones of the same age, grown in glasshouses in soil kept at field capacity, the absorbent surface will be the major important parameter. The stomata respond quickly to environmental conditions so we can measure their resistance to water flow three times a day at 9 AM, 1 PM, 5 PM.

MATERIALS AND METHODS

Our assays were done on two kinds of plants; Poplar (Beaupre, Unal 8) and Azalea (Ambrosius). In both experiments 5 sets of 5 plants each were used. Three checks were used in each experiment; one set received no treatment at all (absolute check), a second was inoculated with the fungus and the third one received only larvae (check for larval mortality without treatment). Two methods of application were tested in each experiment.

We were interested in the plant response to the applied treatment with particular attention to:

1) good control of the pest by the insect pathogen,
2) maximum control before irreversible damage is caused to the plant.

After two weeks, 10 third instar larvae were introduced per plant and 10 ml of conidial suspension of conidiospores (10^8 spores/ml) were poured per 5 litre pot. After 6 weeks, all plants were removed from their pots and larvae were counted.

We used a Delta-T porometer to measure stomatal conductance. A cylindrical cup was temporarily clamped on a leaf. In the head of the cup is a hygrometric film with embedded electrodes. If the cup is swept with dry air, the film is dried. Water is transferred from the leaf to the film which acts as a sink. The electrical resistance of the film decreases. The rate of transfer measures the resistance to diffusion which is dependent on the extent of opening of the stomata.

RESULTS

The first experiment compared the efficiency of fungus application 14 days after larvae or eggs were introduced (on poplar) (Table 1). In the second experiment (using azaleas) the efficiency of immediate (direct) and delayed treatments against third instar larvae were compared (Table 2).

In each experiment, we observed no differences between the check samples. No significant differences in resistance occurred at either 9 PM, 1 PM, 5 PM between the absolute check and plants receiving Beauveria brongniartii alone.

In the plants infested with larvae, we observed a rapid increase in foliar resistance following incorporation of larvae (figure 2). Plants treated with Beauveria brongniartii restored the water balance. However, those plants which received the delayed treatment did not recover completely. The likely explanation of this is that at the time of the delayed treatment irreversible damage had already been done to the plant.

CONCLUSIONS

1. Beauveria activity in vitro was confirmed by in vivo assays. Infection by Beauveria brongniartii in Otiorhynchus sulcatus is reproducible in "horticultural conditions".

2. As few as 2×10^7 conidia per litre of soil kills about 80% of larvae within 21 days.

3. Third instar larvae were easier to use in these experiments but it seems that the first instars were more sensitive. In our test on 600 eggs no larvae were found 38 days after hatching; 46 larvae were recovered in the check test.

4. Leaf conductance is a good non-destructive method to evaluate root damage.

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table 1

experience on Poplar (Beaupre, Unal 8)	check larvae	delayed treatment	check eggs	delayed treatment
O.sulcatus introduced	60	60	600	600
Beauveria brognartii		10 ml		10 ml
number of larvae recovered	48	9	116	0
unmycosed larvae	48	8	166	
Efficency		76 %		100 %

table 2

experience on Azalea Ambrosius	check larvae	direct treatment	delayed treatment
O.sulcatus introduced	50	50	50
Beauveria brognartii		10 ml	10 ml
number of larvae recovered	50	23	32
unmycosed larvae	50	8	14
Efficency		84 %	72 %

figure 1

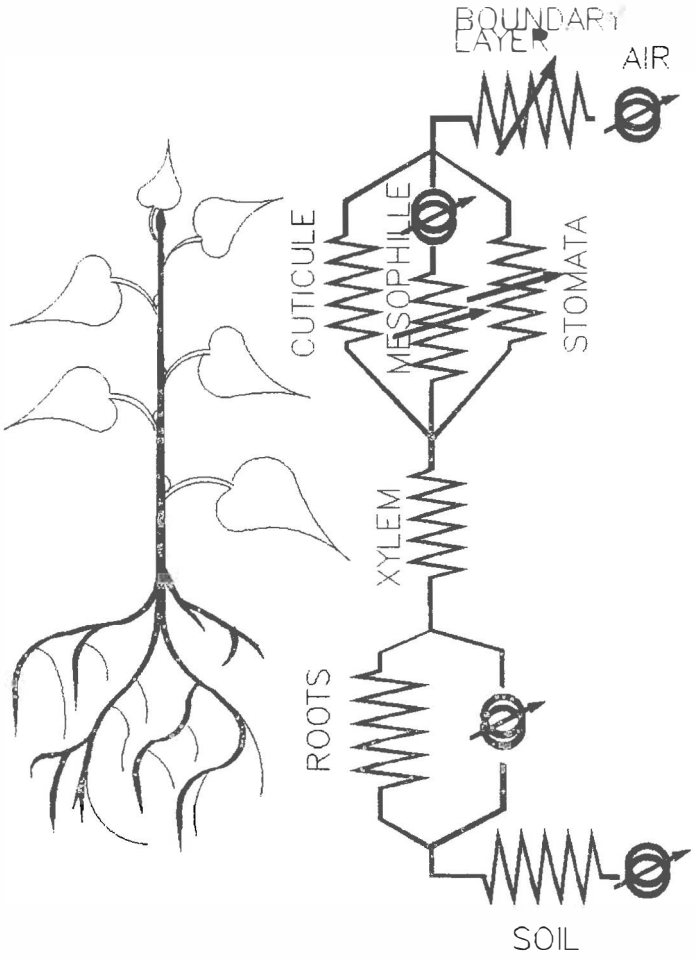
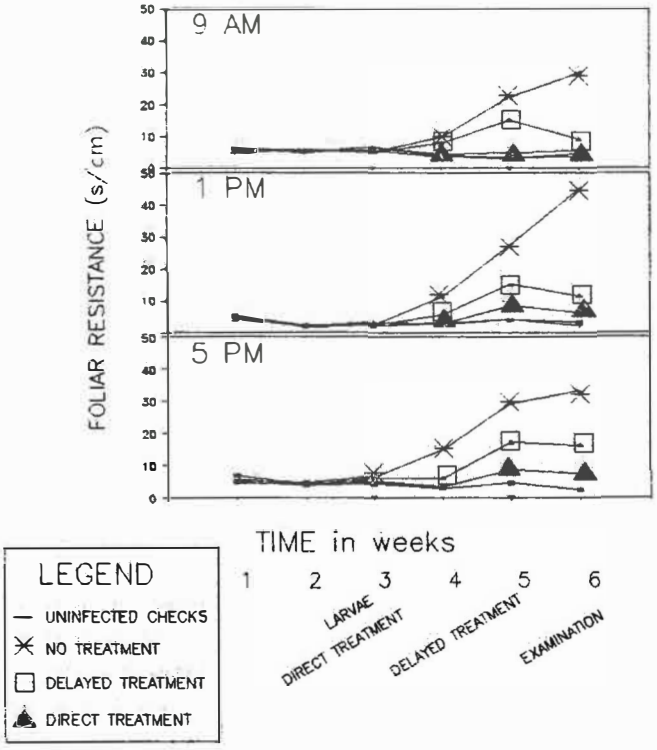

BOUNDARY LAYER
AIR
CUTICULE
MESOPHILLE
STOMATA
XYLEM
ROOTS
SOIL

figure 2 *VARIATION OF FOLIAR RESISTANCE IN RELATION WITH TREATMENT*

experience on AZALEA AMBROSIUS
10 larvae per plant
treatment with 200,000,000 conidia of BEAUVERIA/liter soil



Rapporteur's report

G. RIBA

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Quatre étapes relatives à la mise au point d'un procédé de lutte cryptogamique contre les curculionides ravageurs ont été évoquées lors de la 1ère séance.

1) La prospection de nouvelles souches fut évoquée par tous les intervenants. Soit à partir des populations élevées potentiellement endémiques (MARCHAL), soit à partir de campagnes d'isolements systématiques (KENNETH). La nécessité de trouver des génotypes très différents de ceux que l'on possède déjà fut évoquée par GILLESPIE qui évoque par exemple, en Tasmanie, l'existence de souches eurythermes de M. anisopliae capables d'infecter des insectes à des températures inférieures à 15°C. MARCHAL rappelle qu'il n'y a pas de lien entre la proportion de mycoses observées en quarantaine et le niveau des populations larvaires dans la nature.

PAYNE attire l'attention sur le choix de l'insecte cible qui doit être défini d'une part pour son intérêt agronomique, d'autre part pour sa sensibilité. Un parallèle est alors esquissé entre la fréquence de germes signalés sur un insecte dans la nature et sa sensibilité aux pathogènes (KENNETH). Ce dernier, suite à une question de POPRAWSKI, rappelle les travaux de FARGUES et REMAUDIERE et attire l'attention d'une part sur l'intérêt entomologique de prospections systématiques (nouvelles souches, sensibilité de l'hôte), d'autre part sur son intérêt mycologique (la spécificité d'hôte pourrait être un critère de spéciation).

2) La mesure des dégâts

Les méthodes d'évaluation des populations de curculionides doivent être parcimonieuses car elles sont souvent destructrices. TILLEMANS par mesure du potentiel et de la résistance électrique foliaires propose une méthode précoce et non destructrice de la plante. Ainsi peuvent être visualisées les attaques racinaires dues souvent à la présence de curculionides, ce qui permet une focalisation des traitements sur les foyers d'infection. Avec le même souci, COREMANS-PELSENEER a d'ailleurs montré que des traitements préventifs étaient efficaces.

3) Le criblage de souches

GILLESPIE insiste sur le fait que la présence d'un germe sur un insecte ne signifie pas forcément que celui-la soit pathogène de celui-ci. Pour démontrer la pathogénie d'un germe il faut établir une relation entre la dose et la mortalité. Les techniques de laboratoire mises au point par GILLESPIE ou COREMANS-PELSENEER sont très voisines. Cependant, LATTEUR attire l'attention sur les risques qu'il y a à utiliser des sols autoclavés car la microflore a subi de profondes modifications. MARCHAL ou RIBA utilisent des techniques qui font abstraction de la plante. GILLESPIE qui n'a pas travaillé sur les populations adultes, souhaiterait comparer l'efficacité des souches qu'il a sélectionnées avec celles que MARCHAL a isolées en Bretagne. Enfin, les problèmes techniques liés à l'évaluation d'une population fongique dans le sol évoqués par GILLESPIE, MacCOY et ZIMMERMANN, seront repris dans la séance suivante.

4) L'application

Il faut bien distinguer les cas des ravageurs de culture à haute valeur ajoutée et celui des curculionides ravageurs d'autres cultures. Dans le premier cas, COREMANS-PELSENEER en cultures florales, GILLESPIE et MARCHAL en pépinières évoquent la possibilité d'employer les conidies. Par contre, pour d'autres cultures, seule l'introduction de mycélium déshydraté fixé sur un substrat nutritionnel ("marcescent process") semble agro-économiquement envisageable. A ce propos, MacCOY évoque le risque d'accroître les antagonismes microbiens, tandis que GILLESPIE souligne la latence de ce procédé qui correspond au temps de réhydratation du germe infectieux.

Finalement le sol tamponné, reconnu comme réservoir de propagules ne permet pas une expression agronomiquement intéressante du potentiel infectieux introduit. Si l'utilisation de ces germes dans des cultures à haute valeur ajoutée semble envisageable celui des autres cultures exige des investigations complémentaires. Des problèmes méthodologiques sont soulignés, des collaborations ont été établies ... L'espoir est revivifié !

No abstracts were received for the following papers presented in the session:

Pathogens of Sibona weevils
- J. LIPA

Beauveria bassiana in soils infected with citrus root weevils
- C. McCOY

Some details are given in the following Rapporteur's report.

An additional paper on the use of nematodes was also presented:

Biological control of Diaprepes abbreviatus with Steinernematidae and Heterorhabditidae in the French Antilles
- C. LAUMOND and K. MAULEON

Some details of this presentation are given in the following Rapporteur's report

Rapporteur's report

A.T. GILLESPIE

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This session contained three presentations; from Dr. LIPA, Poland; Dr. McCOY, USA and Drs. LAUMOND and MAULEON, France. The first two presentations were on fungi and the third on nematodes.

Dr. LIPA described an opportunity for using Beauveria bassiana to control weevils, e.g. Sitona lineatus on alfalfa. He appealed for cooperative research in this project as S. lineatus occurs throughout Europe. In E.Europe, alfalfa plantations are maintained for 3-5 years and populations of weevils reach high levels. Repeated pesticide use results in unacceptable residue levels in fodder. B. bassiana is an important mortality factor in weevil populations and can infect up to 60% of S. lineatus. However, in other alfalfa crops the incidence of disease is low, particularly during the first and second years of cropping. A Nosema spp. has also been recorded parasitising up to 20% of a Sitona population. An understanding of the factors governing disease incidence in Sitona populations would possibly allow manipulation of the ecosystem to encourage disease outbreaks and thereby reduce the need for insecticide application. A project is underway in Poland and in Rumania to identify factors governing disease incidence including the effects of climate, fungal population and soil type.

In his paper on citrus root weevils, Dr. McCOY explained that Florida has extensive monocultures of citrus and a complex of important weevil pests; the subtropical/temperate species Pantomorus cervenus and the tropical species Pachnaeus litus, P. opalus, Diaprepes abbreviatus and Artipus floridanus. All these species are univoltine except the latter which has a 90-day life cycle.

A pathogen survey showed a number of organisms caused disease in the citrus weevil complex, including Heterorhabditids and fungi. Paecilomyces lilacinus and Aspergillus ochraceous were found occasionally while M. anisopliae was a little more frequent. However, the commonest pathogen found at all survey sites was Beauveria bassiana. This high incidence, together with commercial interest, the banning of many soil insecticides and the importance of root weevils had stimulated a research programme on B. bassiana.

Before any field testing was undertaken a number of characters were assessed:

1. Biochemical characteristics
2. Pathogenicity
3. Host range
4. Ease of growth relating to production
5. Sporulation
6. Effect of soil moisture on pathogenicity
7. Effect of temperature on spore survival on soils of different moisture levels

Some 38 strains of B. bassiana were examined and all showed similar isoenzyme profiles but comparison with a single Beauveria brongniartii isolate showed distinct differences. Approximately half of the tested isolates had no activity against the weevils and others demonstrated various levels of mortality. The best isolates were RS 252 (from Leptinotarsa decemlineata)

and AF-4 (from a citrus root weevil). LT90 values ranged from 15.2 days after immersion in a suspension containing 5 x 10^6 spores per ml to 3.3 days at 5 x 10^8 spores per ml.

At 30°C AF-4 survived well in soil, in contrast to 40° and 50°C, when viability declined rapidly, particularly in dry soil. Of special interest was the fact that AF-4 was active in over-dried soil (0.5% moisture) while other isolates were poor. Isolates of B. bassiana were of similar pathogenicity in soils containing various levels of organic matter though larvae died more slowly in soils of high organic content.

On the basis of the laboratory data B. bassiana strains AF-4 and RS 252 have been selected for field tests.

This paper demonstrated the many factors which must be considered before selecting a fungal pathogen for field evaluation. In virtually all aspects, strains of fungi vary. It is perhaps better to regard all fungal isolates within a species as distinct, until proven otherwise. Hopefully, by adopting such a patient and thorough approach, more predictable and successful field results will be obtained.

During discussion Professor KENNETH pointed out the records of P. lilacinus as a human pathogen and of the potent toxin produced by A. ochraceous, and then asked how fungal inoculum adhered to insects in soil, and whether B. bassiana sporulated in soil. Dr. McCOY pointed to the work of Boucias where it was demonstrated that conidia adhere strongly to insect cuticle. Dr. McCOY also reported some weak sporulation in soil and this was confirmed by Dr. FARGUES who said that cryoscanning and electron microscopy had showed sporulation of B. bassiana in microcavities in the soil.

Dr.PAYNE asked what soil-type was used to study survival and whether there was any multiplication of the inoculum in the soil. Dr. McCOY replied that sterile soil had been used and that there was an increase in propagule count.

Commenting on the safety of entomopathogenic fungis, Dr. McCOY said that he would not use P. lilacinus and A. ochraceous.

Dr. ZIMMERMANN commented that we know very little about the behaviour of insect-pathogenic fungi in soil. He also commented on the temperature requirements for such fungi. In temperate regions we need isolates with low temperature optima. These would not be of much use in tropical regions.

Dr. McCOY commented that monospore isolates may restrict the range of conditions in which a fungus would be effective and suggested that it would be better to use multispore isolates.

Dr. GILLESPIE mentioned that it was important to select single spores with a range of characters suited to the environment in which they would be used.

Dr. LAUMOND described work on the biological control of Diaprepes abbreviatus with Steinernematidae and Heterorhabditae in the French Antilles. The nematodes studied were a French isolate of Steinernema feltiae (Neoaplectana carpocapsae) and an Argentinian strain of Heterorhabditis bacteriophora. Nematodes were produced in vivo using Galleria mellonella. Young lime plants were grown in plastic bags and each plant infested with a single D. abbreviatus larva.

The most effective treatment was H. bacteriophora applied at 200,000 per plant and only about 10% of larvae were recovered compared to some 60% from the untreated plants. Aldicarb was ineffective in this experiment. S. feltiae was less effective than H. bacteriophora possibly because it was not adapted

to tropical conditions having been isolated in France. The greater control obtained with drenches was possibly due to problems with viability of nematodes produced in Galleria as they may have lost activity during transport. It was suggested that the higher larval populations in the aldicarb treatments might have been due to the chemical adversely affecting natural bacterial and fungal pathogens in the soil.

During discusion it was pointed out that the plant containers were 40 x 10 cm. Dr. POPRAWSKI mentioned that a French isolate of S. feltiae was being commercialised for the control of Otiorhynchus sp.

Round Table Discussion; The use of fungi for control of weevils

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The discussion presented below is an edited version of a tape transcript:

McCOY I wish to turn to topic number one "Methods of pathogen production for microbial control of weevils". I hope certainly our discussion on production can include formulation. Is everyone producing their fungi in the laboratory on a solid based medium? Is there anyone pursuing the use of liquid fermentation in his production method?

GILLESPIE Generally we produce spores (when we need large quantities of them) on barley grain and oil in polyethylene bags and that seems to be adequate for most purposes. But we have recently been looking at the production of conidia of certain isolates of Beauveria bassiana in submerged culture and, at present, we can get yields of around 10^9 conidia per ml. I should say that I am not sure of the properties of these conidia and, in fact, if they are true conidia, but they are certainly pathogenic if not more so than surface-produced conidia. I think, probably, that they are somewhat intermediate between conidia and blastospores and, as such, probably do not store too well. As regards Metarhizium anisopliae, I have looked at that quite extensively over the years and a group at Manchester University has been looking in even more detail and we have not yet managed to get any conidial production in liquid culture at all. You can get up to 10^8 blastospores per ml but that is not economic.

ROSE A long time ago I did some work with B. bassiana and Diabrotica spp., the corn rootworms, which are very important in North America. I think that they are probably number one pests. I did some laboratory work with an isolate of Beauveria which showed some efficacy. I then produced it on oatmeal medium with 10% moisture, in garbage cans sterilized in autoclaves. This produced a nice three-dimensional medium from which I could take the material out, air-dry it and chop it into a granular formulation. So, it was a combination of production and formulation. Fortunately, I was not allergic to Beauveria spore dust because I was covered with it.

FARGUES This question is for Dr.GILLESPIE. You produced conidiospores in submerged culture. But, how did you stabilize this production?

GILLESPIE I think that, in submerged culture, you can get conidia which look to be the same size as vertically-produced conidia. You get blastospores and also an intermediate form, that is, intermediate in size between blastospores and air-produced conidia. I suspect, although I have not looked at it, that the small liquid-produced conidia probably survive quite well but that the intermediate ones probably do not. It is something that needs looking at. In addition, the yields of 10^8 conidia per ml would not be economic. You can get probably 5×10^9 conidia per gram on semi-solid substrates.

McCOY I know of two recent publications on the subject of production of Beauveria in submerged culture. Along with Dr.GILLESPIE's work, one is a recently published paper by a group in Saskatchewan in the Canadian Journal of Microbiology, and another recent paper in this particular area is authored by

Dr.Rombach in the Philippines. So, if you want to expand your knowledge in this area you should discuss with Dr.GILLESPIE and these two people. I believe, Adrian, that in the publication in the Canadian Journal of Microbiology by the Saskatchewan group they identified these intermediate conidia as micro-conidia. Is that correct?

GILLESPIE They did actually observe the intermediate size conidia but they did not make a point of it. They observed that the B. bassiana conidia were produced by micro-cycle conidiation; in some cases, a spore germinates and immediately produces further conidia without any significant mycelial growth.

FARGUES I believe that the form of the conidia is not the real problem. We looked at this for one year in Canada, studying in detail the micro-conidiation in submerged culture of B. bassiana in Goral's medium, Riba's medium and so on. The problem was that, when we studied the survival of these different conidia produced in different submerged culture media, all these conidia survived as well as the blastospores. We did not succeed, with these different forms of conidia obtained in submerged cultures, to increase the resistance to biodegradation in soil.

McCOY Would anyone else like to comment on production as related to submerged culture?

KENNETH I would like to address the gentleman from Ohio (ROSE). He said that he grew Beauveria on oatmeal in garbage cans. You said that you dried it and ground it to obtain conidia. Did you use it in the field?

ROSE It was a granular formulation for corn rootworm control.

KENNETH In other words, it was placed on the soil itself.

ROSE Exactly. Corn rootworms are controlled in North America with granular formulations of insecticides and these are applied at time of planting. This happens to be a very significant market because most of the pesticide companies put all their emphasis onto this. There has been a great deal of resistance development to organophosphorus and organochlorine insecticides such as dieldrin, aldrin and others. It is a very serious problem. It just happens that the farmers have the equipment to apply granular insecticides at the time of planting and the object of our research was thus to produce this microbial insecticide in a formulation that was relevant to what is actually done in the field by the farmers.

KENNETH I attempted something similar about 15 years ago with B. bassiana; my object also was to grind it fine enough to dust it onto wet leaves and to cause sporulation there. The difficulty was to keep the particles on the leaves. They always dropped off after a while. I was told that you need about 40 microns or less in order for these particles to remain on the leaves and I gave up on that. Perhaps somebody would have a better idea but I think that an application technique whereby the fungus will sporulate night after night when you have dews should be sometimes preferable to spraying spores on leaves.

McCOY Is there any other comment about production?

ZIMMERMANN In Darmstadt, we are producing Metarhizium as Dr.GILLESPIE, that is, on cracked wheat or oat kernels in autoclavable plastic bags and this works well, giving a high sporulation. But if we talk about mass production, I believe that we have to think also about the industrial firms because only cooperation with industry will allow us to develop a commercial

mycoinsecticide or mycofungicide. Also, industrial firms have the possibility to produce microorganisms on a large scale. To my knowledge, different industries or firms would like or are able to produce microorganisms in liquid culture using fermentation technology. They have the know-how on fermentation. We should consider how plant pathologists produce their Trichoderma; beneficial fungi which are active against plant diseases. These workers have shown that if you put Trichoderma spores on the surface of the soil they are relatively ineffective but if you provide some nutrients they are very active in the soil. They have thus developed this formulation technique: they pack hyphae in alginate pellets and the fungus develops in the soil from these pellets. I think that this is a quite new idea and that we should try to work out this technique for entomogenous fungi.

GILLESPIE Some of this type of work is being done. Dudley Pinnock, in Australia, has recently produced a patent where he put his Metarhizium conidia in some sort of pellet which rehydrates in the soil, expands and gives significant conidiation. So, I think there is some work being done in that area and I think it is a good area for development, because, if we can get one spore to produce a hundred spores in the soil, then we are improving our economics. In terms of industrial production, I think Abbott Laboratories in the States have attempted or produced B. bassiana on a large scale using rotary drum technology. Any comment Clay (McCOY)?

McCOY I cannot comment on that other than to say that Abbott is producing small quantities of B. bassiana in the U.S. for small field trials. There was a lot of interest expressed by different scientists to obtain a standardized product for testing, so that we can have a better understanding of the performance of Beauveria in one soil versus another soil, say from the north of the U.S. compared to the south of the U.S. We thought that we could accomplish this more effectively if we had a standardized product. We requested of industry that they do this; they agreed and Abbott has been doing it now for about three years. Dr.S.Jaronski is responsible for the overall coordination of this programme. If anyone is interested in small quantities of commercial Beauveria from Abbott, you should write him a letter, specifically explaining your purpose, research-wise, for the requested material. To specifically comment on the methodology used by Abbott or by industry in this particular programme, I must say that I do not know the details on how they proceed with this particular process. I know that there is a solid stage aspect to the production procedure, which leads me to believe that, with Beauveria, they have not developed their technique to the level of sophistication where they are using rotary drums. This is really a tricky method for the Hyphomycetes; when it can be done it cannot always be done successfully, but when it can be done successfully it is very efficient and is probably the best way to go. I do not believe that Abbott has reached that level in their overall production method, but I am not speaking as someone that can read the literature daily to find out what is going on there.

ROSE I want to add one last point concerning industrial production and formulation and the question of actual performance of the fungi. I would also like to say something on the business of safety and allergenicity, particularly respiratory allergy. I think this has been one of the problems with these fungi. Several people I know who have worked with them have been very sensitive to them that is, to Beauveria and Metarhizium, and have reacted very badly. I have worked with them extensively and exposed myself but I have no problems. Maybe it is an individual's reaction. However, since other people have had serious reactions, I think this is a very strong consideration in industrial commercialization, especially concerning the regulatory requirements on the tier system of safety testing that are in effect right now in North America, where a lot of the market is.

LIPA I think that you will be interested to hear about the status of production of Beauveria and some other fungi in eastern Europe. A week ago I visited facilities in three localities in Czechoslovakia where they produce B. bassiana in these so-called sterilized plastic bags on solid medium. In Czechoslovakia, they produce two commercial forms of the fungus, one to be used on field crops, Boverol, and the other developed for control of stored product pests, Boverosil. The conidiospores of Boverosil are stabilized on silica. In the Soviet Union, they produce the fungus by a two phase method: at first in submerged culture and then on a solid medium; the end product is the well known Boverin. Boverin as well as the two Czechoslovakian products are registered. In Poland, my colleagues and especially Dr.Bajan and her group are concentrating their efforts and attention to production by the submerged method, from beginning to end. I was told recently that they were successful in obtaining typical conidiospores, not blastospores. In the production phase, involving the Polish industry, the B. bassiana product will be produced in tanks of up to 20000 litres, thus on a commercial scale. Recent publications on these subjects are "Mycopreparations of the Czechoslovakian Production and Their Use in Protection of Plants" (proceedings of a Nov. 1986 meeting) and the second publication is "Proceedings of the Nitran, Czechoslovakia, Symposium on Biological Plant Protection, 24-26 August 1987". Both books have some chapters dealing with the industrial production of B. bassiana.

McCOY I want to make a general comment on the importance of stability of any fungal preparation, particularly Beauveria, in terms of any field work. There is well documented information in the literature that shows that stability is a very important factor to consider in any production programme. So, a good bioassay technique is a very important aspect of this overall approach to produce the fungi as microbial control agents. If I may lead to that particular point, I think it is appropriate at this particular time to have a general discussion of bioassay techniques. Does everyone feel comfortable with their bioassay methodology as it relates to weevils?

GILLESPIE I think there is an urgent need for a suitable bioassay system for vine weevils and other weevils. Your (McCOY's) assay system seems interesting. Perhaps that could be adapted for use with the vine weevil. Do you maintain the larvae without any soil?

McCOY The larvae of the root weevil that we are working with are produced on an artificial diet but, as I pointed out, we do use neonate larvae. What we basically do is collect the larvae at the time that they hatch from the egg; we take larvae that are no older than 48 hours. We put them into the holder in the presence of the spore suspension; the suspension is agitated for one minute and then put on the filtration device which draws the fluid across the integument of the larvae which are then transferred to the culture units with or without a source of food substrate. We found that the food is not really that critical. The important thing is to keep the organisms on a moist filter paper during the time that you are incubating them until you are ready for the bioassay.

GILLESPIE Your control mortalities in that system are very low?

McCOY Yes, they are low, very low.

PAYNE Has anybody apart from yourself (McCOY) developed satisfactory rearing systems, in vitro systems, artifical media, for any of the weevils we are talking about in western Europe?

McCOY No. In vitro rearing of many weevils presents many challenges. Any other comments in terms of bioassay? Another suggested topic for discussion was "Survey for useful pathogens", is enough being done?

BURGERJON What about the Bacillus thuringiensis strain which is specific against beetles? Is there potential for this strain? Maybe our colleagues from Darmstadt can answer the question.

POPRAWSKI I can tell, that in the States, Mycogen and Ecogen, two small private firms, are deeply involved in researching the potential of this new strain of B.t. against the Colorado potato beetle. There is a market there.

BURGERJON Does it work?

POPRAWSKI This I do not know.

ZIMMERMANN In Darmstadt we have tested the activity of this new strain of B.t. against Otiorhynchus larvae and we did not get any effect. It seems that this strain is restricted in activity to the Colorado potato beetle although you have some secondary hosts such as leaf beetles. Some leaf beetles are very sensitive but it is a question of the concentration of B.t. that you use in the tests. Again, the Colorado potato beetle is the most important susceptible host from the agricultural point of view.

GILLESPIE Is it necessary to look for new strains of fungi? I think that the danger is that we have so many strains of fungi that we do not know anything about. I think of the new ARS-USDA collection catalogue which has some 3000 fungal isolates in it. I guess we know very little about more than 1% of those strains. So, I think maybe it is more important to learn more about the strains we do have than to go collecting others. Although, maybe for a specific pest in a specific area of the world it is worth collecting to see what is in that natural environment. But there appears to be isolates from virtually all the orders of insects in the collections these days, and they are all available.

PAYNE We have heard from Dr. KENNETH that he and Dr.I.Ben-Ze'ev prepared a list of Entomophthorales which apparently infect Coleoptera. Do we have this information for weevils and Deuteromycetes?

KENNETH No and I think that we should. This was my plea today that somebody should take this under his wing. It might take more than one person. It certainly should be an entomologist, an entomological taxonomist who could recognise changes in the genus name of an insect for instance. I think that overlooked "ancient" literature should be searched for, as one can be surprised just how excellent, exact and dependable some of that old, perhaps discarded, literature can be. A complete compilation should be made, and then also compared with the Entomophthorales-on-Coleoptera list. I am certain that they will come up with some very interesting information, of practical value as well.

McCOY Is anyone working with Paecilomyces fumosoroseus as a pathogen of weevils?

GILLESPIE We examined a strain of P. fumosoroseus and one of P. farinosus; they were very low in activity against the vine weevil and we ditched them in favour of M. anisopliae.

McCOY This discussion relative to the fungi has focused on Beauveria and Metarhizium; it looks like M. anisopliae is most studied in soil versus weevils and others. Is there a reason for that choice?...SILENCE...Apparently not.

PAYNE Can I broaden that point and ask if there is any evidence that any of these fungi are perhaps better than others in establishing in the soil? Are we going to apply these fungi regularly for biological control or can we expect them to establish and persist for long periods?

McCOY Certainly someone should be able to comment on that. At this point, may I ask if anyone has reported epizootics associated with these fungi?

POPRAWSKI Yes, Aeschlimann reported Beauveria epizootics in Sitona populations in southern France some 4-5 years ago.

RIBA It was 5 years ago. In my opinion the problem is not the presence or not of one species (of fungus) but the presence or not of one strain.

FARGUES I agree with Dr. RIBA. When we studied the persistence of different strains of various species of Hyphomycetes, when we compared B. bassiana strains, M. anisopliae strains, P. fumosoroseus and P. farinosus strains, we found large differences in survival in soil between the different strains of the same species. For example, we have a strain of M. anisopliae that disappeared in six months when applied to soil as a conidial inoculum and we have another strain where the inoculum remained at the same level for more than two years. Previous studies in Darmstadt have demonstrated that Metarhizium in soil was better than Beauveria, but our experiments showed that it is more important to consider the strain than the species.

McCOY Does anyone have something to add to these comments?

COREMANS-PELSENEER In our experiments in glasshouses, we always use the same Beauveria brongniartii strain against Otiorhynchus. When we treat, we put conidia at the same time, on soil with plants and in sterile soil. After the experiment, we look at the number of viable conidia. Today, five months after the first experiment was set up, we can still detect the fungus in both soils.

McCOY Any comments relating to strains of Metarhizium or Beauveria?

KENNETH In regard to strains, I have never heard of anybody showing interest in the ability or inability of conidia of hyphomycetous entomopathogens to germinate on a leaf surface. I do not know how it is with Metarhizium or Beauveria but with Nomuraea rileyi there are conidia of some strains which would not germinate on leaves on which I tested them, yet I have heard of germination of other strains on leaf surfaces. If, during a single dewy night, all conidia germinate on a leaf, then most would be wasted. Once the germ tube is out, it is out and is likely to die if it has not reached the target insect. I would suggest looking for a strain of Beauveria or Metarhizium or Paecilomyces or the like which will stay quiescent and hopefully alive on the leaf until the insect appears.

McCOY My experience with Beauveria is that it will not germinate in water. Is this unique to my strain(s) or do you all find that in distilled water you cannot get them to germinate?

COREMANS-PELSENEER I observed this. In water, even incubated at 25°C, only 0.5 to 5% of the spores will germinate, but if you place them on a moist filter paper in a petri dish, most will germinate. They will also germinate readily if you add carbon or a source of carbohydrates.

McCOY Or a source of nitrogen.

KENNETH Perhaps there is a difference depending upon which leaf surface the conidium falls because there are exudates from some leaves, including carbon and nitrogen compounds. In some of these leaves there may be enough to cause the spores to germinate. Perhaps there is some level of nutrients below which there will be no germination.

FARGUES I believe that the problem is not the same if we consider persistence on foliage and persistence in soil. In soil, the persistence depends on the ability that a conidium has to persist, yes, but it seems to me that it depends mainly on the ability of the conidium to undergo microcycles. Indeed, when we monitor an inoculum for two years in soil, I do not believe that the spores we recover at the conclusion of the experiment are the same spores that we placed in the soil two years earlier. Our hypothesis is that there must be microcycles in the soil, and we have observed in vitro microcycles with some strains of fungi. On foliage, in the climatic conditions of France or the U.K., the persistence of spores ~~is better~~ if we deal with quiescent spores than if we have spores germinating on the surface of the leaves since hyphal forms are more susceptible to environmental factors.

McCOY To further comment on this topic of strain selection as it relates to soil microorganisms, do you see here Dr. PAYNE, an opportunity for cooperative research? It is pretty obvious from the discussion that we had today that there are many factors associated with soil. Do you see common denominators from one area of research to the other that may be important both from the stand point of using a specific strain or representative groups of strains as they relate to the control of particular soil weevils.

PAYNE I think it would be very useful if, in any future cooperation in this area, a consistent bioassay can be carried out. For example, Dr. COREMANS-PELSENEER indicated that she is doing work on persistence of fungi in sterile sand. I think in this particular case, that people who are studying persistence and establishment of fungi in soil could, maybe, at least evaluate their strain using a common technique as well as their own variation of that technique. Ideally, we are hoping to look not at sterile situations but at the world of the soil and everything it contains. But, on the other hand, it would be very useful to know how people's individual strains stand up to, for example, the sort of bioassay system that Dr. COREMANS-PELSENEER is using. We need to standardize a bioassay technique.

McCOY I think this is a very good point to make because as we all know from the reported literature, particularly, as it relates to human pathogens, it is important that we recognise that the soil system itself has a very definite effect on the overall defence mechanism associated with various host systems. To address carefully the variation that you are interested in genetically, it is extremely important that the sensitivity of the bioassay be known. For example, what is the difference between using sterile and non-sterile soil?

COREMANS-PELSENEER We need to label or mark strains, especially of B. brongniartii, used in bioassays with weevils in soil. We do it with sterile soil to be sure that the strain we take out is the same we put in. In sterile soil, of course, you are sure, you have a check, but this is not true for non-sterile soil.

MARCHAL C'est d'autant plus intéressant que, vraisemblablement, dans le sol, pour un même genre de champignon on doit avoir beaucoup plus d'espèces que l'on pense. Dans certaines populations d'insectes il n'y a pas, vraisemblablement, un seul B. bassiana ou B. brongniartii mais il y a plusieurs B. bassiana ou B. brongniartii et il doit en être de même pour la plupart des genres de champignons. Jusqu'à présent on ne caractérise pas de façon systématique chaque souche de champignon isolée d'un insecte en particulier ou de plusieurs insectes, d'un même genre d'insectes et d'une même espèce d'insectes issus d'un même site. Tant que l'on aura pas fait cet effort on ne pourra pas dire, sauf si l'on utilise en effet des souches marquées, ce qui se passe dans le sol, même en conditions très contrôlées. On ne pourra que spéculer. Il serait donc intéressant de créer un groupe de

spécialistes qui s'attacheraient particulièrement à l'étude de ces phénomènes dans le sol et le cadre de l'OILB me semble bien indiqué. Il pourrait en être de même pour l'ensemble des problèmes Curculionidae, non seulement pour Otiorhynchus, mais aussi pour d'autres curculionides européens et du nouveau continent.

McCOY Any other comment related to the biological control of weevils or on the importation of new natural enemies? If not, let us move to the next topic "Integrated pest management programmes against weevils". How do we use these organisms in an IPM system? We should address now the utilization of chemical fungicides. What do they do to our fungi? Are we looking at the necessity of developing fungicide-resistant strains along with all the other attributes that we do worry about?

GILLESPIE The effect of fungicides on Metarhizium or Beauveria is important and needs to be looked at and perhaps resistance to fungicides is a useful method of marking the strains we put into the soil. This would enable us to recover them and might also provide for possible patents.

McCOY Is anyone in this group working in this area of research, that is the development of fungicide-resistant strains? I think if someone has such a strain he will be very popular. Any further comments on resistance, selection of resistant strains both as markers or to improve resistance?...SILENCE...I think of one area that became very evident to me today in the presentations and certainly reflects a very positive aspect of this workshop, I mean the interaction of nematodes and fungi as natural control agents, particularly of the larval stages of weevils. Is there anyone working on this from an IPM standpoint? Is there antagonism, is there synergism? I know from our initial studies in Florida that it appears that the fungi were active earlier in the year and then the nematodes came in and became predominant. I do not know what happened at the time that the interaction took place and have no idea. Anyone with comments in terms of interaction between entomophilic nematodes and entomogenous fungi, specifically relating to the weevils we are talking about today?

DESEO We have some experience, not with weevils but with tree borers. We have found that a specific strain of B. bassiana mixed with nematodes is very effective against the goat moth (Cossus cossus); the mixture gave at least 20-50% better results than nematodes alone. We did thus improve the effects of the nematodes with conidia of B. bassiana produced on Sabouraud maltose agar; we carried out the experiment in an apple orchard where we applied the mixture with an atomizer. Results were very good.

McCOY Any other comments on fungi-nematode interactions?

PAYNE If the nematodes work as well as we have seen today in so many of the talks given, why work on fungi? Do fungi have any advantages in production, specificity, levels of control, that we cannot achieve readily with nematodes?

GILLESPIE I think that fungi are probably as easy and cheaper to produce than nematodes in the current state of the art.

McCOY Is that all? We people working on fungi have the opportunity to defend ourselves here.

ZIMMERMANN I think there is in fact a kind of competition between nematologists and entomopathologists but our work comes together. The advantages of the fungi are, perhaps, that they may persist longer in the soil and, perhaps again, that they may suppress beetle populations at a lower level and for longer periods of time. In this case, nematodes and fungi might be combined; the nematodes for rapid depression and the fungi for longer term activity.

DESEO This is just what I wanted to say. In the case of tree borers, we have used the nematodes as vectors. Nematodes entered the small holes and killed some of the tree borers and the Beauveria conidia carried on the cuticle of the nematodes infected and killed the remaining larvae of the leopard moth. So, I think that we can ~~increase~~ the low mortality caused by the nematodes by combination with special strains of Beauveria. For example, a Czechoslovakian strain of Beauveria did not work against the goat moth, but our special strain was all right.

ZIMMERMANN One main advantage of the nematodes is that they do not have to be officially registered and this means that small firms can produce them and sell them directly to private users. Entomopathogenic fungi need registration, they must pass the safety tests, and all this adds to the costs of production.

McCOY In the U.S. nematodes are exempt from the EPA review process, fungi are not.

PAYNE In the U.K. we felt that this was going to be the situation as well. It may still prove that it will be so but we do face problems at the moment with the Department of the Environment in relation to the use of non-indigenous nematode strains. Two to three years ago, a Tasmanian strain of Steinernema was introduced and used in a glasshouse and mushroom house experimentation for control of vine weevils and one or two other pests, and it was used without, apparently, the need for registration. But it has been pointed out that, in the U.K., the Wildlife and Countryside Act of 1979 or 1980 does not permit us to introduce non-indigenous strains; and when we say strains, I mean strains which, in biological terms, we cannot yet discriminate between. This attitude has largely been stimulated by a greater appreciation by legislators in the country to the problems associated with non-indigenous organisms and genetically-manipulated organisms. Whether this approach will spread to other countries I do not know, and to what extent it will continue to give us problems in the U.K., I do not know either.

McCOY This is a key topic. Does anyone else, from industry let us say, want to comment on that aspect?...SILENCE...If not, is there anything else, in particular relative to the fungi, that anyone wants to bring to our attention?

KENNETH I want to stand up and to speak for the fungi since their value has been questioned. I believe that fungi are so diverse that we have not really scratched the surface when it comes to their possibilities. They have sometimes attributes that we have not quite even thought of utilising. For instance, Dr.DESEO has just mentioned the leopard moth and nematodes. Leopard moth would be the last insect that I would consider to utilise fungi against. Yet ants having travelled through some fungi will introduce the spores into the burrows of the leopard moth, to the very end where the larvae are and the fungi will kill them. That is a rather surprising thing. Nobody thought about that until a colleague, Mr.Y.Nakache, did, and proved it experimentally. I am not saying that we should start growing ants and utilising them but I am showing you the diversity of means, and the surprises that are in store for us; if we keep at it we eventually are going to make break-throughs on this particular subject.

(SMITS) I do not want to attack fungal people, but can you show us one example anywhere in the world of a consistently successful fungal use, as an insect parasite or in any other way, against any pest insect or pest organism? My opinion about fungi is that they are always so unpredictable and will stay so for ever.

PAYNE Having asked the question, perhaps I should say that there are good examples of success; I think of citrus white flies and Aschersonia as a classical example of long term biological control by a fungus and probably one of the earliest examples of classical biological control. I think we also have examples of Erynia species controlling pests in orchards. I cannot remember what particular pest, but once various fungicides started to be used in the orchards, then the problem of the insect pest became a considerable problem.

GILLESPIE Another couple of examples: there is an Israeli isolate of Zoophthora radicans introduced into Australia that provided partial control of the alfalfa aphid and in Brazil about half a million hectares of sugarcane are treated every year with M. anisopliae and that has been going on for about ten years now.

McCOY It is quite evident from the comments that have been made today, we are dealing with a management-type system and, as we learn more about how these microorganisms are interacting in the soil system, we are going to better utilize them in management systems in the future. In most situations, no one knows the true benefit of soil pesticides for control of these weevil pests in terms of efficacy and effect on natural enemies. So much needs to be done. Thank you everyone for attending.

ENVIRONMENTAL PERSISTENCE OF PATHOGENS

a) Fungi

ETUDE DE LA PERSISTANCE D'HYPHOMYCETES ENTOMOPATHOGENES PAR INTRODUCTION DE GENOTYPES ETRANGERS A L'AGROSYSTEME

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La faible persistance des hyphomycètes entomopathogènes est en contradiction apparente avec leur ubiquité et l'existence d'épizooties. Nous avons décidé d'introduire, en régions tempérées, des germes différenciables des souches sauvages dans des contextes agronomiques où de fréquentes muscardines ont été observées. Dans le cas des populations d'**Otiorhynchus sulcatus** en fraiseraies bretonnes, plusieurs espèces sont responsables des mycoses d'insectes, alors que dans le cas des populations beauceronnes de la pyrale du maïs **Ostrinia nubilalis**, seul **Beauveria bassiana** affecte les chenilles. Le mutant de **Beauveria brongniartii** résistant au bénomyl a été retrouvé sur l'ensemble des insectes atteints de muscardine.

De même, après son introduction dans le couvert végétal, le mutant de **B. bassiana** résistant à la fois au bénomyl et au fenproprimorphe, a tué seulement 38 % des larves au cours de l'été mais il fut responsable de 61 % des cas de muscardines blanches développées au cours des quarantaines des chenilles diapausantes. Par contre, ce germe n'a eu aucune incidence sur la génération suivante. Une observation comparable a été effectuée par suite de l'introduction de la souche N° 78 de **Metarhizium anisopliae** qui est pathogène pour les chenilles de la pyrale du maïs. L'application massive de cette espèce, (10^{13} sp/ha), rigoureusement étrangère aux cultures de maïs en France, a induit un effet choc qui réduisit de 43 % l'effectif larvaire estival. De plus 56,5 % de chenilles diapausantes ont été tuées par ce germe au cours de quarantaine. L'identité du cryptogame a été vérifiée par analyse électrophorétique de quelques systèmes isoenzymatiques. Cette importante activité ne s'est pas maintenue l'année suivante puisqu'à l'automne seulement 8,8 % des chenilles diapausantes meurent de muscardine verte.

Dans l'ensemble de ces observations, et grâce au suivi spécifique d'un germe de génotype identifiable, les auteurs concluent (1) qu'une importante proportion des larves édaphiques d'Otiorhynque ou de pyrale sont au contact d'hyphomycètes entomopathogènes , (2) que l'introduction massive de **B. bassiana** ou **M. anisopliae** dans le couvert végétal peut induire une sensible réduction des populations estivales de la pyrale du maïs, (3) qu'en dépit de ces deux constatations favorables le genre introduit affecte peu ou pas les générations suivantes.

Les auteurs attirent l'attention sur la nécessité d'une étude approfondie du passage des propagules infectieuses entre les phases aérienne et édaphique.

Microclimatic studies of the persistence of Nomuraea rileyi

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Field persistence of conidia of Nomuraea rileyi (F.) SAMSON was studied at the top of the vegetation in order to evaluate the influence of solar radiation. Field trials were conducted on pigeon bean (2 experiments) and on cabbage (1 experiment) in 4 m^2 plots. Four configurations were tested; they consisted of (1) one plot covered with a screen eliminating direct sunlight (RYT^-), (2) one plot covered with a glass screen transmitting solar radiation from 320 to 2 500 nm ($UV\ A^+$), (3) one plot covered with a glass screen coated with a UV A and B blocking film cutting wavelengths above 400 nm (UV^-) and (4) one uncovered plot exposed to direct sunlight (290 to 2 500 nm) (RYT^+), respectively. The estimates of N. rileyi survival were based mainly on viable conidia counts and in the cases of both (RYT^+) and (RYT^-) configurations, the pathogen activity was assayed on larvae of Spodoptera littoralis BOISD. Logarithmically transformed viable spore counts were analysed using a linear model and results were expressed in terms of the half-life of viable conidia. Micro-environmental parameters monitored in the field included sunlight, hours of sunshine, air temperature, leaf surface temperature, relative humidity, leaf wetness duration and precipitation.

The half-life of viable spores appeared to be dependent on the sunlight intensity. Under sunny conditions the half-life decreased to 3.6 hr, but when plots were covered with a screen blocking the direct sunlight (RYT^-) it could be of 40 hr or more. The data of pathogen activity (angular values) of the spores over time declined like their viability.

The use of selective screens transmitting the UVA radiation (UVA^+) or blocking the wavelengths above 400 nm (UV^-) demonstrated clearly the lethal effect of solar UV A radiation on the spores deposited on leaves exposed to direct sunlight in the field. For example, the half-life of conidia could be reduced 4 times when exposed to UV A (x $UV\ A^+$ = 11.6 hr for x UV^- = 48.2 hr).

A predictive equation was formulated and it was speculated that in very sunny conditions a high, concentrated inoculum of N. rileyi viable conidia (3×10^6 spores/cm^2) could be reduced 10^4 times over 7 days, whereas it decreased only 100 times during cloudy weather.

Considering 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. Spore inocula, deposited on cellulosic supports, were exposed to different ranges using Schott filters cutting radiation at specific Wavelengths (WG 280, WG 300 and GG 400). For instance, the WG 280 filter transmitted radiation above 280 nm with a UV B density (280 to 320 nm) of 2.5 W/m^2, a UV A density (320 to 400 nm) of 23 W/m^2 and a total flux density (300 to 3 000 nm) of 360 W/m^2. Comparative measures of sunlight irradiance in La Minière in June at 12.00 hr gave the following data 2.0 W/m^2 (UV B range) 40 W/m^2 (UVA range) and 800 W/m^2 (near UV to near IR). Moreover, in order to investigate the heating and the lighting effects of visible and infra red radiation, inoculum supports were put on a heat-regulated plate.

The germination rate and viability (colony forming units) of irradiated spores dropped very rapidly, within approximately 20 min, when exposed to radiation including UV B. A great amount of inactivation occurred within 100 min in the inocula exposed to radiation above 320 nm (including UV A). Within the duration of exposure, visible and near infra red radiation did not induce any lethal effect on N. rileyi conidia. On the other hand, after 10 hrs of exposure to radiation above 400 nm, a decay of spore viability was noted. Thus the inoculum was entirely killed within 40 hrs. In darkness, the viability of N. rileyi spores remained at the original level for at least 5 days.

Furthermore, results showed that sublethal doses of UV A delayed the germination process. This retardation could reach 90 hrs.

These experiments under controlled conditions confirm our previous results on the influence of UV radiation and particularly of UV A radiation on N. rileyi field persistence. Concerning the other ranges of wavelengths, it was established that the viability of N. rileyi conidia depends also on the light and heat effects of visible and infra red radiation.

A microclimatic approach to the short term persistence of pathogens in plant canopies is necessary to facilitate an evaluation of the individual as well as the combined effects of environmental factors. A better understanding of field persistence of pathogens depends on interdisciplinary efforts devoted to build explanatory and predictive models. Experiments under controlled conditions on the effects of abiotic factors enable us to elaborate submodels based on biological phenomena. In the case of modelling the influence of sunlight on short-term persistence, these submodels could be coupled with microclimatic models of sunlight penetration into the vegetation.

ENVIRONMENTAL PERSISTENCE OF *BEAUVERIA BASSIANA* IN THE SOIL

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In view of the limitations being placed on chemical insecticides for control of hypogean insects, the need for biorational soil insecticides such as entomogenous fungi is greater than ever. Many laboratory and field trials with *Beauveria bassiana* indicate high potential for control, yet application often fails. The stability of entomopathogenic fungi is known to be affected by physical factors such as exposure to sunlight, improper moisture conditions, temperature extremes, substrate chemistry, and chemical antagonists introduced by man and produced in the soil by microbes (Roberts and Campbell, 1977; Tedders, 1981; Lingg and Donaldson, 1981). Obviously, one or more of these factors can effect the survival of either indigenous or exotic isolates of *B. bassiana* in the soil.

Such limitations may be overcome by selection of isolates resistant to the particular factors important in limiting survival in tropical soils. Preliminary research suggests that exotic isolates of *B. bassiana*, highly infectious to many citrus root weevils, have distinct temperature and moisture tolerances in different soils (McCoy et al., 1985).

In Candler type soil, 4 isolates of *B. bassiana* (252, 143 mono, AF-4, and 738) applied at 1×10^7 conidia/ml of soil were tested for infectivity to larvae (48 mg $\pm$ 20) of *A. floridanus* at 0.5, 5.0, 17.0,

Migration and persistence of Metarhizium anisopliae in the soil

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Metarhizium anisopliae is a widely distributed and well-known entomopathogenic fungus which is currently used for biological control of soil-inhabiting insects. Besides other factors, its efficacy in the soil greatly depends on the migration and the persistence which subsequently are affected by several abiotic and biotic factors, such as soil moisture or water, soil texture and soil organisms. In the following experiments we have tested the vertical movement of M. anisopliae conidia suspended in 0,05 % Tween 80 using soil columns and two different, nonsterile standard soils as well as its migration and persistence in the field. For reisolation of the fungus, the soil dilution plate technique and the following semiselective medium was used: OAES-(Ohio Agric. Exp. Sta.) Medium, 5g glucose, 2g yeast extract, 1g $NaNO_3$, 0,5g $MgSO_4$. $7H_2O$, 1g KH_2PO_4, 1g oxgall, 1g Napropionate, 50mg streptomycin sulphate, 50mg chloramphenicol, 20g agar, 1000ml dist. water, autoclaved at 105°C for 15 minutes. In both standard soils, the majority of M. anisopliae was recovered in the upper 10-15 cm of the soil columns. However, differences occur with respect to soil type. In the humus-poor sand no conidia could be detected in depths from 15 to 30 cm and in the effluent; in the humus rich, loamy sand, however, conidia moved deeper and in some experiments they were recovered even in the effluent. In the field, a spore suspension and fungus overgrown oat kernels were applied separately on the soil surface. In the following three years, propagules of M. anisopliae could be detected regularly in different soil depths up to 20 cm. Conidia suspended in a wetting agent (Tween 80) percolated deeper than such from oat kernels.

PARAMETRES MICROCLIMATIQUES ET APPAREILLAGES SPECIFIQUES UTILISES SUR LES DISPOSITIFS DE TERRAIN PENDANT LES CAMPAGNES D'ETUDE DE LA PERSISTANCE DU GERME *Nomuraea rileyi* EN 1984 et EN 1986.

(ROUGIER, M. & FARQUES, J.)

PARAMETRE	VARIABLE	APPAREIL	CAPTEUR (x)	TYPE DE MESURE (y)	NOMBRE DE CAPTEURS 1984	1986
Température	Sol (température de référence)	Canne de sol (1,20 m de profondeur)	Thermocouple K Sonde de platine	Ponctuelle Continue	1 (a) 1 (a)	1 (a) 1 (a)
	Air	Prise ventilée	Thermocouple T	Continue	1 (a)	1 (a)
	Feuilles	Pinces-prototypes Radiothermomètre	Microthermocouple T Capteur à infrarouge	Continue Ponctuelle	1 (b) 1 (a)	3 (b) 1 (a)
Humidité relative	Température air et HR %	Psychromètre automatique type SECK et PERRIER	Thermocouple T	Continue	1 (a)	1 (a)
Température du point de rosée	Température du point de rosée	Hygromètre d'ALLUARD		Ponctuelle	-	1 (b)
Mouillage	Durée d'humectation	Kit humectographe	Prototype INRA-STEFCE	Continue	1 (b)	2 (b)
Vent	Vitesse m/s	Anémomètre MCB		Continue	1 (a)	1 (a)
Rayonnement	Global (400 - 1200 nm) Wm^{-2}	Pyranomètre LAMBDA	Photodiode au silicium	Continue	1 (a)	1 (a)
	UVA et UVB Wm^{-2}	Radiomètre UV CENTRA	Photodiode au silicium	Ponctuelle	-	1 (b)
Insolation	Durée (heures)	Héliographe de CAMPBELL et STOKE		Continue	1 (a)	1 (a)

(a), (b) : capteurs utilisés sur l'ensemble du dispositif (a), ou par microparcelle (b)
(x) : thermocouples : type T = cuivre-Constantan ; type K = chromel-Alumel
(y) : tous les appareils de mesure en continu sont connectés sur une acquisition de données JOSY 800.

and 20.0 soil moisture levels. Isolate AF-4, 252, and 738 were infective at all moisture levels while isolate 143 mono produced only 20% mycosis at 0.5% moisture level (dry) and was least virulent at other moisture levels after 12 days. AF-4 was most virulent at the 0.5% moisture level causing 100% mycosis after 6 days. AF-4, 252, and 738 all gave greater than 87% mycosis at 12 days at all moisture levels. The LT_{90} for isolate AF-4 at 1 x 10^8 conidia/ml of Candler soil was 7 days. B. bassiana isolate AF-4, indigenous to Florida soils, appears to be most virulent at all moisture levels.

The pathogenicity of the 4 isolates of B. bassiana (252, 143 mono, AF-4, and 738) were also compared at 3 dosages (10^6, 10^7, and 10^8 CFU/ml) in non-sterile organic (Zellwood muck) and sandy soils (Wauchula, Candler, and builders sand). Phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) levels were very low in the Candler and builders sandy soil (< 10 ppm) compared to Zellwood muck and Wauchula soils (> 88 ppm). Soil pH ranged from 6.0 to 6.8.

In all pathogenicity tests using 35 to 45-day-old laboratory-reared Artipus floridanus larvae for bioassay, LT_{90} values decreased with an increase in dosage (5-7 days at 1 x 10^8; 10-14 days at 1 x 10^6). No apparent difference in LT_{90} was detected among fungal isolates for each soil type. LT_{90} values for organic soil or sandy soil with high P, K, Mg, and Ca were similar to sandy soils with low soluble salts.

In 4 independent field studies conducted during the spring and summer of 1986 in 2 central Florida citrus groves infested with either Diaprepes abbreviatus or Pachneaus litus, a commercial formulation of B. bassiana (ABG-6178/252), was applied as a drench treatment to the soil surface over the root zone of the tree (16 sq. ft.) for larval control.

The fungal pathogen was applied at rates of 1, 10, and 100 g per 16 sq. ft. to regularly irrigated soil. A standard chemical treatment (Mocap and/or Aldrin) and an untreated check were included for comparison. Survivorship of the pathogen in the soil was monitored using 3 methods: a) laboratory bioassay where defined soil samples from 3 depths (0-2", 4-6", and 10-12") from each treatment were exposed to 30-day-old laboratory-reared larvae for 7 days, b) field bioassay where 30-day-old laboratory-reared larvae confined to screen cages were buried in treated soil in the field for 7 days, and c) soil samples from 3 depths were processed as washes and serial dilutions drop-plated on a selective media for B. bassiana. Adult weevil/tree and adult weevil emergence from cages were determined throughout the season too.

Results from all field studies showed that:

a. Monitoring methodology employed for detecting B. bassiana persistence in the soil was both sensitive and accurate.

b. Artificial inoculation of irrigated soils with B. bassiana resulted in a significant increase in propagule density (1×10^6 CFU/cc) and larval mycosis (70-80%) compared to the untreated check with 1×10^1 CFU's/cc and 0-5% larval mycosis. Both fungal propagule density and larval mortality declined gradually following application.

c. A positive correlation was found between propagule density of B. bassiana in the soil and root weevil larval mycosis.

d. A minimum dosage of 100 g/16 sq. ft. of B. bassiana with a potency of 2×10^{10} CFU/g is required to achieve a soil propagule density of 1×10^6 CFU/cc of soil and 70 to 80% mycosis of 30-day-old weevil larvae. This is equivalent to 60 lb per treated acre.

e. Fungal propagule density and larval mycosis were highest at the upper 2 inches of soil.

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The survival of inoculum of conidia of *Erynia neoaphidis* Remaud. and Henn. on unsterilized soil.

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Summary

The conidium of Entomophthorales is a thin-walled asexual spore which germinates to form a germ tube able to penetrate the insect cuticle and cause infection or a conidiophore bearing a secondary conidium which, in its turn, becomes the infection unit. Higher order conidium may form subsequently.

The results of a study on the persistence of the production of replicative conidia of an inoculum of primary conidia of *E. neoaphidis* laid on the surface of a wet or dry loam at 20° C and on a wet loam at 5° C are presented.

In wet conditions, samples incubated at 20° C produce replicative conidia during 24 days. The results suggest that most secondary conidia that form, at least after the initial 24 h. period, do not project a new conidium.

When soil samples were dried immediately after they were inoculated with primary conidia and moistened 4, to 7 days later, 5-10 times more replicative conidia were produced during the first days after the samples were moistened than during the same period from samples kept moist throughout.

The ability to produce replicative conidia is conserved for 6-8 months at 5° C. However, the longer the period in these conditions the shorter the period during which replicative conidia are produced when the samples are subsequently incubated at 20° C.

The duration of the period over which replicative conidia are emitted from the soil may explain how species which do not produce resting spores, such as *E. neoaphidis* overwinter. This also allows some latitude for the encounter between host and pathogen.

Mortality caused by Beauveria bassiana (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) in overwintering populations of Xanthogaleruca (=Galerucella) luteola Müll. (Coleoptera: Chrysomelidae).

ORESTE TRIGGIANI

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ABSTRACT

Xanthogaleruca luteola Müll. (Coleoptera: Chrysomelidae) a pest of the elm trees is widespread in Europe, Asia and North America causing leaf damage as larvae and adults along with the deterioration and even death of trees.

Observations made during the autumn and winter months of 1985-1987 in Apulia (Southern Italy) indicate that B. bassiana is the only but very important factor of mortality of X. luteola adults overwintering under the bark of eucalyptus and grape vines.

The specific character of bark on the eucalyptus trees provides more favourable microclimatic conditions for the survival and development of B. bassiana while the higher populations of insects favour the spread of infection.

The mortality rate of adults overwintering on the eucalyptus reached in February of 1987 the level of about 87% while on the grape-vine the level of 67%.

Rapporteur's report

G. Latteur

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EXPOSE DE G. RIBA Pas de question.

EXPOSE DE J. FARGUES

QUESTION DE B. PAPIEROK Y a-t-il sur le feuillage une différence de persistance entre les conidies de Nomuraea rileyi produites in vitro et in vivo?

RESPONSE L'etude a été exclusivement réalisée avec des conidies produites in vitro et nous ne pouvons donc pas répondre à cette question. Cependant, des expériences ont été réalisées dans le sol avec des conidies de N. rileyi produites in vitro et in vivo et on a constaté que les premières survivaient moins longtemps que les secondes.

QUESTION DE McCOY Selon la littérature, l'activité de N. rileyi est très variable selon les souches. Ce champignon est un pathogène qui agit loin du sol plutôt que dans le sol ou il hiverne dans le sol. Selon vous, y a-t-il des souches qui prédominent surtout au-dessus du sol plutôt que dans le sol?

REPONSE Le travail que nous avons effectué est un modèle réalisé avec cette souche sur le feuillage car elle est pathogène de Spodoptera littoralis qui est l'insecte ciblé. Nous n'avons cependant aucune information sur son comportement dans le sol et je ne puis donc donner un avis à ce sujet.

EXPOSE DE G. ZIMMERMAN

QUESTION DE B. KENNETH Avez-vous utilisé du sol non stérile et pensez-vous que la pénétration dans le sol de conidies sèches (hydrophobiques), non produites en présence de Tween 20, ne serait pas moins importante que celle de conidies qui, comme les vôtres, ont été produites en présence de Tween 20?

REPONSE Le sol utilisé était non stérile. Nous avons commencé à réaliser le même type d'expérience avec des conidies non traitées au Tween 20 et nous avons effectivement constaté que leur pénétration était moindre, de l'ordre de quelques centimètres.

EXPOSES SUIVANTS Pas de questions.

ENVIRONMENTAL PERSISTENCE OF PATHOGENS

B) Other Pathogens and Nematodes

Enhancement of *Bacillus thuringiensis* and *B. sphaericus* Persistence with Slow-Release Formulations

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Bacillus thuringiensis and *B. sphaericus* are of major significance among microbial pesticides because they are both produced by submerged fermentation with cost/effectiveness comparable to chemically derived pesticides and they are safe to nontarget organisms, man and the environment. They are selective in action and manufactured with renewable agricultural resources. However, they are usually nonpersistent in performance with some exceptions and usually require frequently repeated or carefully timed applications to control target pests. Repeated applications increase labor and equipment costs and critical timing requires surveillance expertise and costs to determine the most appropriate time for application. Extra costs for repeated treatments reduce cost/effectiveness and make it economically desirable to prolong the period of control. Delayed-release or long-term control formulations are a practical way to enhance their useful persistence in the environment.

In 1976, a new type of *B.t.* was found in Israel that is effective on mosquito and black fly larvae. It was named variety *israelensis* and serotyped H-14. Commercial efforts to prolong the time of performance or persistence resulted in briquets or doughnuts made of a composite of cork granules and *B.t.* H-14 powder which gives mosquito larvae control for up to 30 days or more depending on their circumstances of use. Slow-release floating pellet, tablet, wafer, biscuit or briquet formulations can be made using various floating particulate materials bound by water soluble adhesives in a floating matrix with *B.t.* H-14 or *B. sphaericus*. They may also be made by attachment or inclusion of a float of balsa wood, cork or other natural or synthetic flotation material to a nonfloating matrix containing the microbial pesticide. Floatation can further be obtained with CO_2 produced by bread yeast or baking powder in the matrix. Slow-release occurs by natural erosion of the floating matrix which allows active particles to persist in the feeding zone and settle to the bottom over an extended period of time. Another approach is to combine the microbial pesticide with monomolecular film to keep it at the water surface as long as possible.

For Lepidoptera caterpillar control, there have been different efforts to obtain more persistent formulations of *B.t.* variety *kurstaki* serotype H-3a3b and others. Bran and other carriers have been used as the basis for noctuid control baits. Microencapsulation has been tried, but is generally expensive. Ultraviolet light inhibitors or masks have also been used with some success. Good agricultural sticking agents in the formulation or spray tank mixtures are possibly the most beneficial persistence enhancing agents because they adhere the *B.t.* particles to plant surfaces and help prevent rain or dew from washing them off.

More research and development needs to be done to enhance the performance of microbial pesticides with more persistent formulations to improve their cost/effectiveness which is often a major criteria in user/farmer acceptance.

Persistence to washing with rain of Bacillus thuringiensis and virus inclusion body preparations

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Different preparations of Bacillus thuringiensis (B.t.) were subjected to the action of a stream of rain water ("washing dose") in a laboratory treatment tower (Burgerjon, 1964). This washing affects, to a greater or lesser extent, the deposit of the active material, the physical persistence of which is evaluated by bioassay, which permits the checking of the residual activity of the different preparations. Several "washing doses" were tested on cabbage leaves fed to caterpillars of Pieris brassicae. The deposit of a preparation based on B.t. spores and crystals persists to a much greater extent after washing with rain water than a preparation based on the soluble heat-stable toxin (beta-exotoxin). Deposits of different formulations of spores and crystals also show considerable difference in persistence.

Using the same laboratory treatment tower, it appears that the activity of dried spray deposits on pine needles of inclusion bodies of Thaumetopoea pityocampa cytoplasmic polyhedrosis virus are not affected by substantial rinses (Burgerjon and Grison, 1965).

Dried spray deposits on apple leaves of a Cydia pomonella granulosis virus preparation (Carpovirusine) are not washed off by tap water spraying on apple trees in the orchard nor by rinsing in a stream of water (Burgerjon and Sureau, 1985).

Both the crystalline parasporal bodies of Bacillus thuringiensis and the virus inclusion bodies are proteinaceous and this may explain the excellent resistance of dry deposits to rain washing. The water soluble heat-stable toxin of B. thuringiensis has no such resistance, although the same toxin is insoluble in some preparations (Burgerjon, 1967). Research workers using Carpovirusine unnecessarily reapplied treatments after some rainfall until the persistence experiments decribed here were carried out.

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Acquisition and Loss of Nuclear Polyhedrosis Virus Inclusion Bodies by Plant Surfaces

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This study deals with some aspects of the quantitative relationships of the polyhedral inclusion bodies (PIBs) of nuclear polyhedrosis viruses (NPVs) with plant surfaces and has been approached through observations relating to three main questions:

1. Have particular NPV's coevolved with particular plants so as to be acquired and retained by their surfaces and is this especially true where the insect host is of a very plant specific nature? The study employed the NPV of the *Pinus* specific *Neodiprion sertifer* (NsNPV) and the NPV of the polyphagous *Spodoptera littoralis* (SlNPV). Needles of *Pinus contorta* and leaves of *Gossypium hirsutum* were dipped for one minute in suspensions of Ns and SlNPV (2.5×10^8 PIBs/ml) in deionised water, and were then rapidly washed for either 5 or 60 seconds in 200 ml of water. PIB counts were then made on upper and lower leaf surfaces using an SEM. PIBs were acquired by leaf surfaces but had a skew distribution and therefore before analysis counts were transformed according to Taylor's simple power law. There was much significant variation within treatment replicates but little significant variation between upper and lower leaf surfaces on 5 and 60 second washes for either plant species. However, NsNPV was acquired more readily by both *P. contorta* and *G. hirsutum* than SlNPV, suggesting it to be the more 'avid' PIB. SlNPV was acquired more readily by cotton than by pine, possibly indicating a degree of poor adaptation to the latter leaf surface. Despite apparent differential avidity, the level of loss (%) between 5 and 60 seconds wash was fairly constant.
2. It is known (earlier unreported work) that PIBs can be very rapidly acquired by hydrophilic plant surfaces: but can they also be similarly acquired by hydrophobic surfaces? To test this, interveinal strips of *Brassica oleracea* leaves were fastened to microscope slides which were then inclined to such an angle (80-85°) that a falling 20µl drop (containing 6×10^6 SlNPV PIBs/drop) would traverse their length with great rapidity. The leaf strips were not visibly wetted during drop passage, but nevertheless to remove "loose" PIBs were immediately washed for 5 or 60 seconds. Leaf sectors were then inspected by SEM and PIBs counted on standard areas. PIBs were retained where the leaf surface was smooth but not where there was epicuticular crystaline wax. Apart from this, PIBs were present along the full length of the droplet track.

3. What are the rates of natural attrition of purified and unpurified preparations of PIBs from coniferous leaf surfaces? Foliage of *Picea abies* and *Picea sitchensis* was dipped in aqueous suspensions of either pure or impure *Gilpinia hercyniae* NPV and of *P.sylvestris* in pure or impure NsNPV. Using a sticky tape leaf surface stripping method (Elleman *et al.*, 1980), PIB samples were taken at weekly intervals for 50-200 days according to treatment. The decay curves are essentially semi-log normal. The rate of loss (sequential half-lives) tended to be faster first and slower later. Loss was significantly faster for purified PIBs.

Discussion

Long range forces (mainly electrostatic; van der Waals, hydrophobic interactions etc.) are involved in acquisition and shorter range forces (various types of chemical bonds) in retention of PIBs on leaves (Small, 1985).

It appears PIB acquisition by leaf surfaces can be very rapid (e.g. a spray droplet rolling over the hydrophobic leaf of *B.oleracea* may leave many PIBs behind) and that it may also be preferential (e.g. NsNPV was acquired more than SlNPV). The forces causing physical attrition have not been closely investigated but may include rainfall and epicuticular erosion by dust (Jones *et al.*, 1987). On conifers, impure deposits persist better than pure PIB deposits, possibly because of adhesive contaminants. As has been shown, however, (Evans and Entwistle, 1982) comparatively brief periods of PIB persistence may not be epizootiologically important if larval corpses retained on plants continue, by slow decay, to supply PIBs to foliage between generations of susceptible host larvae.

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Persistence of virus on pine foliage over winter

J.S.Cory, W.R.Carruthers and P.F.Entwistle

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As part of field trials in northern Scotland designed to look at the feasibility of using a nuclear polyhedrosis virus (NPV) to control pine beauty moth (Panolis flammea), the persistence of the virus on the foliage over winter was studied together with its effect, if any, on the next season's P.flammea generation.

One hectare plots of lodgepole pine (Pinus contorta) had been sprayed with P.flammea NPV early in the summer of 1985 and the resultant infection monitored until the larvae pupated in August. Foliage was collected from one of these plots during the winter months and again in the following spring to ascertain whether viable virus was still present. As the foliage could not be bioassayed directly with the insect, virus was recovered from the foliage samples using a sonication technique. The viral material was counted and then bioassayed in an alternative host, the cabbage moth (Mamestra brassicae).

Infective virus was recovered from the foliage up until the hatch of the 1986 larval generation. Countable quantities of viable virus were found on the new 1985 foliage as well as on the one year old foliage. Plots sprayed in 1985 were monitored during the following larval season to see if any viral infection was present in the populations in the plots. The number of larvae found in the plots was low, a result of the successful 1985 spray trials; nevertheless, low levels of viral infection were diagnosed in both late instar larvae and in pupae.

Determining virus persistence: The case of *Spodoptera littoralis* NPV on cotton in Egypt.

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Since 1979 TDRI has undertaken an extensive research programme into the production, formulation and use of *Spodoptera littoralis* NPV for pest control in Egypt. As part of this programme we have carried out a detailed study into the persistence of this virus on cotton. The purpose of this was to determine the relative importance of the various environmental factors on virus persistence under conditions in which it will eventually be used and from the results make recommendations for formulating the virus for field use.

In common with other studies, sunlight was shown to be the most important factor determining the inactivation of virus in the field. It was shown that the ultraviolet portion of sunlight, in particular wavelengths from 305 to 320nm, accounted for most of the inactivation observed.

Field temperatures, humidity and the effects of cotton leaf exudates were found to cause no significant inactivation over the period measured (up to 7 days). However, physical loss of virus from the leaf surface was found to be important and this accounted for up to half of the loss of activity noted in the field.

Persistence was found to be considerably longer on the undersurface than on the upper surface of leaves. This was due to shading from UV radiation and because the rate of physical loss was slower from the undersurface of the leaves. The longer persistence on the undersurface of the leaves is particularly important as it is this region that the target insects (1st instar larvae) feed.

Unpurified virus was shown to be more resistant to UV inactivation and to physical loss than purified suspensions.

This study emphasised the need to determine virus persistence in the field if a formulation is to be designed that will protect the virus against the environment in which it is to be used.

Activation of an apparent latent virus in *Pieris brassicae*

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Throughout the literature on baculovirus infections there are many instances cited of larvae succumbing to viral disease which is claimed to be the result of activation of "latent virus". If latent viruses are as widespread as is suggested by all these reports, then it would appear to be a major factor in the environmental persistence of many baculoviruses. Unfortunately, very few investigators have fully characterized these viruses or attempted to explain in what form the virus may remain latent within the insect. In many instances it is quite likely that infection is due to contamination of the insect - the method of induction of the disease, e.g. heat, cold, chemicals, etc., having merely served to lower the susceptibility of the insect to viral infection.

In studies on the host range of *Artogeia rapae* granulosis virus (ArGV) it was found that although two very closely related isolates (ArGV1 & 2) were moderately infectious for *Pieris brassicae*, most isolates (ArGV3 - 15) were able to cause only low levels of infection even at extremely high doses. Virus was individually purified from *P. brassicae* larvae which died following high dose infection with ArGV5 & 8 and the DNA from each preparation was analysed with several restriction enzymes. This showed that:

1. There was great variation between the virus isolated from different larvae.
2. None of the RE-patterns matched the profiles for ArGV5 or 8.
3. A number of the profiles were similar to that for ArGV1.
4. All the profiles contained several bands present only in ArGV1 but some also contained a number of bands present only in ArGV5 or 8.

A comparison of the physical maps for ArGV1 & 5 and progeny viruses suggested that the latter resulted from recombination between the inoculum ArGV5 and viral DNA which was identical or very similar to ArGV1. This ArGV1 DNA could originate from: 1. contamination of the virus inoculum; 2. contamination of the insects; or 3. a persistent or latent infection of the insects.

A number of factors, which will be discussed, suggest that contamination is a very unlikely explanation for the results obtained. We therefore conclude that 1. *P. brassicae* carries a latent or persistent infection. 2. Recombination can occur between an inoculum virus and the latent virus giving rise to many new genotypes. 3. Although the inoculum dose in these experiments was high, it represented only 0.1% of the virus from a single infected larva. Such doses may therefore be acquired naturally and give rise to recombinant viruses in the environment.

COMPARATIVE FIELD PERSISTENCE OF GRANULOSIS VIRUSES UNDER TROPICAL AND EUROPEAN CONDITIONS

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A main problem in the use of insect viruses for pest control is that their persistence in the field is restricted by environmental conditions. It is well known that the ultraviolet radiation of the sun is an important factor in inactivating baculoviruses. The UV stability of viruses in the field has been widely studied, but most of these trials have been carried out in temperate climates (JAQUES, 1977; RICHARDS and PAYNE, 1982). Only little is known about the inactivation of baculoviruses in tropical areas where the incidence of the UV radiation is rather high. The aim of this study was therefore to compare the field persistence of two viruses, the granulosis viruses of the false codling moth, Cryptophlebia leucotreta, (ClGV) and of the codling moth, Cydia pomonella, (CpGV) under different climatic conditions.

The experiments with the ClGV were carried out on the Cape Verde Islands, an archipelago in the Atlantic near Africa, situated at the 17^{th} parallel of latitude. Here, as well as on the African continent, the false codling moth is a serious pest in citrus, avocados and other fruit cultures (FRITSCH and HUBER, 1986). In order to examine the inactivation of virus deposits on the foliage, in October and November 1986, trees on a small citrus field were treated with the ClGV. Using a hand operated pressure sprayer, several small trees were sprayed to run off with virus suspensions containing 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 virus granules/ml. As a sticker 1.0% skimmed milk powder and as a wetting agent 0.025% CITOWETT was added to the spray. In the same way apple trees on the experimental field of the Institute for Biological Pest Control in Darmstadt had been been sprayed with the CpGV in summer 1981 and 1982. During all the experiments the insolation was recorded with a glass-ball heliograph.

The virus activity on the foliage was determined by sampling leaves from the treated trees at intervals of several days after the virus application. The leaves were assayed in the laboratory following the method decribed by KRIEG et al. (1980): disks were cut out of the leaves and fed to neonate larvae of Cryptophlebia leucotreta and Cydia pomonella. The larval mortality was recorded after 7 days. Since several gradual virus concentrations have been applied in the experiments, for every sampling date a concentration-mortality curve and the corresponding LC_{50} values could be calculated. The reciprocal value of the LC_{50} gave a direct measure for the viral activity remaining on the leaf surface. For short periods of exposure the relationship of the logarithm of the activity and the time, measured in number of days or sunshine hours, resulted in a linear inactivation line.

Former trials had already shown that under changing weather conditions, the number of sunshine hours are a more adequate measure for the exposure time than the number of days. The data in table 1 seem to confirm this for the trials carried out in Darmstadt, particularly if one considers that the intensity of the UV radiation decreases with the decreasing height of the sun in the second half of the year. On the Cape Verde Islands on the other hand, the half-life of the virus activity seemed to be rather independent from the mean number of sunshine hours per day; in both experiments its value was close to 2 days. This is probably caused by the fact that the UV radiation in the tropics was high also under a cloudy sky. The trials in Darmstadt and on the Cape Verde showed that during sunny weather the inactivation rate was rather similar on both locations. But under cloudy conditions the virus persistence was much better in the temperate climate, where the half-life of the virus deposit was nearly a week.

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table 1: half-life values for the field activity of the ClGV on the Cape Verde Islands (CV) and the CpGV in Darmstadt, Germany (DA)

trial	average sunshine-hrs/day	height of sun at noon	half-life days	half-life sunshine-hrs
CV 07.10.-21.10.1986	2.3	68° - 62°	2.2	4.5
CV 11.11.-29.11.1986	6.0	56° - 52°	1.9	12.2
DA 07.07.-03.08.1981	3.7	62° - 57°	6.5	20.0
DA 21.06.-20.07.1982	6.9	63° - 60°	2.0	14.3

Mobility and persistence of entomoparasitic nematodes in field conditions

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The vertical and horizontal mobility, as well as the persistence of a few nematode species in a sandy clay loam (sand 47%, clay 25%, silt 28%), have been observed in outdoor conditions in 1986/87. Nematodes were applied on 13 October 1986. One million juveniles of each species (Heterorhabditis heliothidis, Steinernema glaseri, S. feltiae strain "1192") were distributed by a watering pot on 1 sq metre plots either previously irrigated or not. By the means of augers, 30 mm in diameter, 20-25 cm long cylindrical samples of soil were collected at intervals over a period of 203 days. The samples, divided into 3 cm sections, were tested by Galleria-trap for the presence of nematodes. The eventual horizontal mobility of nematodes was checked 145 and 203 days after distribution.

The results of the trial are summarised as follows:

1) Vertical mobility: H. heliothidis and S. feltiae moved downwards very quickly; they were detected 1 day after distribution at a depth of 20-25 cm, while S. glaseri was within 10 cm of the soil surface. The downward movement of H. heliothidis and S. glaseri seemed not to be affected by previous irrigation, while in the case of S. feltiae it seems that the irrigation favoured the distribution, due to differences in depth in which juveniles were observed in the irrigated and non-irrigated plots.

2) Horizontal mobility: no nematodes were detected in the soil samples taken outside the plots after 142 days, while only H. heliothidis was found 40 cm from the plot 203 days after application.

3. Persistence: H. heliothidis was by far the most persistent, being detected in many samples even 203 days after distribution. The presence of S. feltiae "1192" was detected for the last time (and even then in only a few samples) after only 64 days.
Since S. glaseri persisted for only 9 days, it was re-applied on 7 November 1986. However, also in this case, it persisted for only a short period.

Environmental Persistence and Population Dynamics: The Results of Mathematical Models

Dr. M. Begon, Dept. of Zoology, Univ. of Liverpool, U.K.

This presentation is, in large part, an exercise in communicating the results of Anderson & May (1981), as they relate to the environmental persistence of pathogens, to an audience of non-mathematicians and perhaps non-quantitative ecologists. However, some of Anderson & May's assumptions are questioned, and the conclusions drawn do not, therefore, always coincide with theirs.

In the present context, the mathematical approach can be used to address a number of related questions.
How do different patterns of environmental persistence influence host-pathogen population dynamics?
What types of population dynamics might we expect to arise from the patterns of environmental persistence that we think exist?
What are the main characteristics - with respect to environmental persistence - required of a pathogen if it is to be able to control a given pest species?

Possible patterns of environmental persistence can lead to a wide range of dynamical behaviour - from no effect, through a reduced rate of host population growth, to a stable, reduced abundance of the host, to cyclic variation in abundance and prevalence. However, for baculoviruses at least, cyclic variations seem not to be as important as Anderson & May suggest, since the effective number of infective particles produced at the death of an infected host (i.e. the number of particles with at least a moderately long life-expectancy) is not infinitely large (Anderson & May) but often quite small. Indeed, the most likely combinations of parameter values are in a region where any of the four patterns of dynamical behaviour are feasible.

Moreover, from a practical point of view, it would seem not so important to emphasise the pattern of dynamical behaviour (as Anderson & May do), but rather to concentrate on the level of host abundance around which fluctuations (or no fluctuations) occur. This level depends on the rate of production of infective particles (Λ), on their mortality rate (μ) and on their transmission coefficient (ν) - the rate at which they successfully infect hosts. These three exert their influence as the following compound parameter: $\frac{\mu}{\nu\Lambda}$

and successful (usable) pathogens are characterised by low μ and/or high ν and/or high Λ.

This conclusion is somewhat unremarkable, but it does
(a) focus the mind on the fact that low μ and low ν (particles in refuges 'protected' from mortality factors and uninfected hosts) and high μ and high ν tend to be associated with one another, whereas what we require is low μ and high ν; and
(b) indicate that control strategies may be judged in terms of their relative effects on the three paramters, even where estimates of their absolute values are impossible to make.

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RAPPORT DE LA PREMIERE TABLE RONDE DU SYMPOSIUM

"Persistance des germes pathogènes et des nématodes parasites d'insectes"

Jeudi 3 septembre, 14h 50 - 16h 30

Animateurs: Jacques FARGUES et Bernard ITIER

Rapporteur: Bernard PAPIEROK

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En préambule, J. FARGUES énonce les différents points devant être abordés au cours de la table ronde et ses objectifs. Il donne ensuite la parole à M. BEGON, qui présente l'approche mathématique de R.M. ANDERSON et R.M. MAY (1981) dans la modélisation de la dynamique des populations d'un parasite et d'un invertébré-hôte, en s'attachant particulièrement à l'aspect: persistance dans l'environnement. L'orateur discute des 3 paramètres conditionnant la persistance d'un agent pathogène: nombre de particules produites, leur taux de mortalité (ou mieux de survie) et leur coefficient de transmission (pourcentage de réussite de l'infection). Il discute également de la mesure de ces paramètres, ainsi que de la notion de seuil de densité de la population-hôte en-dessous duquel l'agent pathogène est incapable de persister. En définitive, les modèles mathématiques devraient constituer un support permettant d'apprécier, relativement, l'efficacité d'une stratégie de lutte par rapport à une autre.

A l'issue de cet exposé, Brigitte GAUX soulève le cas des modèles décrivant l'évolution, en fonction des facteurs abiotiques, des paramètres conditionnant la persistance. M. BEGON souligne alors la complémentarité de ces modèles et de ceux qu'il vient de présenter. Pour M. MARCHAL, ces modèles apparaissent restrictifs: le cas envisagé est celui d'un pathogène spécifique et d'un hôte donné. L'éventualité d'une spécificité peu étroite chez le pathogène (comme chez certains Hyphomycètes) et celle de l'existence d'hôtes de remplacement devraient être prises en considération. De son côté, P.F. ENTWISTLE envisage le cas où l'hôte a la possibilité de quitter le biotope et donc de ne plus être au contact du pathogène: il importe par conséquent que les modèles soient les plus larges possible. En conclusion de cette discussion, J. FARGUES insiste sur le fait que les modèles dont il est question concernent la persistance des agents pathogènes à long terme; aucun ne traite de la persistance à court terme.

M. ROUGIER présente ensuite les méthodes utilisées par l'INRA-La Minière pour la mesure des paramètres microclimatiques, le plus souvent en continu et sur de longues périodes. Certains paramètres sont mesurés par des capteurs classiques. Pour d'autres, il a fallu mettre au point des capteurs particuliers (capteur à pinces à thermocouple dans le cas de la température de la feuille) ou adapter des capteurs du commerce (mesure des UV solaires). Les conditions d'utilisation de ces méthodes ont été déterminées. Suite à l'intervention de K.A. JONES se rapportant à la méthode qu'il a utilisée en Egypte pour mesurer l'UVB sur feuille de coton, B. ITIER souligne la nécessité de séparer le facteur rayonnement du facteur température et les problèmes posés par l'hétérogénéité au niveau du couvert. Il est ainsi amené à insister sur l'intérêt qu'il y a à développer des observations en chambres climatisées. M. ROUGIER présente alors les résultats obtenus dans de tels dispositifs expérimentaux. Il en ressort notamment une bonne corrélation entre l' UVA et l'UVB d'une part, et le rayonnement global d'autre part. La mesure de celui-ci suffit donc pour obtenir une appréciation de l'UV. L'analyse de l'effet des radiations est réalisée à l'aide de plaques thermorégulées.

A l'issue de l'exposé de M. ROUGIER, C.C. PAYNE demande si les capteurs utilisés sont disponibles ou encore au stade de prototypes de laboratoire. Un point a également son importance, c'est celui de l'angle d'incidence du rayonnement solaire sur le support. A ce sujet, il serait utile de s'informer auprès de G. TOMKINS de l'USDA à Beltsville (Maryland, Etats-Unis d'Amérique). P.F. ENTWISTLE remarque qu'il serait particulièrement opportun que quelqu'un s'attache à rédiger un document précisant les procédés actuellement disponibles de mesure des UV et d'interprétation des données.

J. FARGUES donne ensuite la parole à C. MCCOY qui présente, dans les grandes lignes, les problèmes posés par l'existence dans le sol d'un complexe d'antagonistes, qu'ils soient corps chimiques ou organismes vivants. Considérant notamment l'action éventuelle de champignons sur les nématodes s'attaquant aux insectes, C.C. PAYNE signale l'intérêt qu'il y aurait à établir sur ce sujet une collaboration avec le groupe de travail de l' OILB/SROP s'intéressant aux microorganismes et aux insectes du sol. G. ZIMMERMANN souligne à son tour l'étendue des problèmes relatifs aux phénomènes d'antagonisme intervenant dans le sol. Le champ de recherche apparaît extrêmement vaste. Ainsi, des champignons entomopathogènes pourraient avoir une action vis-à-vis de champignons phytopathogènes. Le genre Hirsutella contient à la fois des espèces s'attaquant aux nématodes et des espèces entomopathogènes. De son côté, R.G. KENNETH signale la richesse et la variété des organismes vivants dans le sol. Pour G. RIBA, il importe d'étudier la réceptivité des différents sols aux champignons entomopathogènes, en s'inspirant notamment des recherches déjà menées dans ce domaine avec les champignons phytopathogènes. Des programmes de collaboration devraient être mis en place et l'on gagnerait à étudier les acquis de la microbiologie du sol. Enfin, Jacqueline PELSENEER émet l'hypothèse que des organismes vivants dans le sol pourraient se comporter non pas en antagonistes, mais en éléments susceptibles de favoriser l'action des pathogènes.

Au terme de cette table ronde, R.G. KENNETH trace le portrait d'Isaac HARPAZ qui était attendu à ce colloque, et qui vient de disparaître. R.G. KENNETH rend hommage à l'éminent scientifique mais également à l'homme érudit et cultivé qu'était cet ancien doyen de la Faculté d'Agriculture de l'Université hébraïque de Jérusalem.

Workshop 2: Environmental Persistence of Pathogens. 2nd Round Table Discussion

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The second roundtable of the workshop on environmental persistence of pathogens was chaired by P.F. ENTWISTLE. Its main topics were:

- **persistence of pathogens and measures for protection aginst inactivation (continuation from first roundtable)**

- **the phenomenon of latency or inapparent infections.**

In addition to that, vivid discussion circled around the two questions:

- **Does microbial control have a potential for industrialized countries or is it only feasible in the 3rd World?**

- **Is lethality the one and only criterion for the efficiency of a microbial pest control agent?**

In the following lines an attempt is made to summarize the discussion about these four topics:

1) Environmental persistence.

The session was opened by the presentation of a paper by J.S CORY et al. on the "Persistence of virus on pine foliage over winter" which reported about the persistence of the nuclear polyhedrosis virus of Panolis flammea from one season to the next on pine trees (for details see abstract above).

Then W. KRIEG presented some data on the effect of rain on the persistence of Bacillus thuringiensis tenebrionis in the field. Contrary to the findings A. BURGERJON had reported in the morning session, he measured a very quick loss of endotoxin activity on the foliage, which he attributed to the washing off of the toxin crystals. The discussion following the paper gave no clue for the possible reasons of the discrepancy of the results. Unfortunately, due to the rainy summer in 1987, KRIEG was not able to have a trial without rain, which could have served as an untreated control.

In the second part of his paper, KRIEG showed results of laboratory trials on UV-inactivation of B.t.t.. The radiation of an artificial UV-source (xenon-lamp) had a pronounced effect on the activity of the preparation, which was reduced by the addition of riboflavin or folic acid. These findings were difficult to explain since - as the discussion showed - on the one hand, the spores do not play a role in the efficacy of B.t.t., and on the other hand it is generally assumed that the endotoxin is not susceptible to UV radiation. In the discussion it was suggested studies be conducted on the effect of UV on protein as there seem to be some diversity of opinion in this field of research. Generally there seems to be a lack of information with regard to the influence of spray physics on the susceptibility of the resulting spray deposits to UV and on the efficacy of UV protectants. The behaviour of the pathogen particles or the dilution of the UV protectant in the spray droplets spreading on the leaf surfaces may be of importance. Therefore, the spraying techniques best suited for chemical pesticides (e.g. controlled droplets) are not necessarily also the best for microbial preparations.

In view of an apparent need for such sophisticated research, the provocative question was raised, whether it was worth the labour just to end up with

50 to 100% improvement of persistence. The discussion showed that prolonged persistence is needed not so much for better efficacy but to have more redundancy and reserve in the treatment, making, e.g. the timing of the application less critical. There are other methods to achieve this goal: several participants in the discussion reported on the successful use of frequent applications of low dosages (examples: codling moth granulosis virus; Verticillium lecanii against white flies; Heliothis NPV).

Many problems regarding selection of application technology and persistence of spray deposits could be solved by more critical studies on the behaviour and biology of the target insect. This statement, brought forward by the chairman, was subtained by several examples from the audience and met general agreement.

2) Latency

In his introductory lecture, N. CROOK summarized literature data and his own results, indicating that the phenomenon of latency might be quite common with baculoviruses. Probably a considerable number of transmission results in the older literature has to be attributed to the activation of latent viruses. Unfortunately, in most cross transmission studies, the viruses have hardly ever been characterized biochemically. For the nuclear polyhedrosis virus of Autographa californica, e.g., about 40 successful transmission studies have been reported in literature. In only 5 of them was the identity of the virus in the new host verified with DNA analyses. Though CROOK judged the possibility of exogenous infection by contamination in his own trials to be very unlikely, many of the seemingly sucessful activation of "latent" viruses in the past have to be attributed to contamination of laboratory and equipment by the virus. Several participants in the discussion illustrated this by examples from their own trials. Unfortunately, this kind of data is hardly ever published. Spontaneous virus infections usually occur as a nuisance in experiments designed for other purposes and are rather unpredictable and difficult to repeat. More well documented data, which include also biochemical characterisation of the viruses, are badly needed.

3) Significance of microbial control in industrialized and in third world countries.

In view of the difficulties encountered when trying to integrate microbial methods into pest control strategies in industrialized countries on the one hand, and the success microbial control agents have in the third world on the other hand, the provocative suggestion was made to restrict research in microbial control to third world pests. This opinion was vividly contradicted by the audience. It is true that the numbers of insect viruses which have potential for commercialization is rather limited. On the other hand, alternatives to chemical pesticides, particularly environmentally acceptable ones, are badly needed, even more so in industrialized countries. Microbial preparations may also give relief in situations where increasing resistence in the pest insects reduces the efficacy of chemical insecticides.

In spite of its present successes, microbial control in the third world cannot give a lead in the use of microbials in pest control. Actually, in the sixties, before more strict registration protocols came in use, viral control had a similar boom in the industrialized countries as now in the third world. Nowadays, field use of insect viruses will not be permitted by registration authorities without a vast body of data on their impact (or non impact) on the entire ecosystem. Cottage type production methods in use in the third world will also not be acceptable to commercial companies in industrialized countries. So both approaches have their merits and should be pursued, benefitting one from the other through extensive transfer of knowledge.

4) High lethality as a prerequisite for a microbial control agent.

There was general agreement in the audience that high insecticidal activity is not necessarily needed for good efficacy of a microbial in pest control. On the contrary, one of the most successful viruses ever used in biological control programmes, the nonoccluded baculovirus of the rhinoceros beetle, Oryctes rhinoceros, gives greatly delayed lethality. Its main feature is that it reduces the fertility of the beetle drastically. A pathogen which does not kill its host immediately may even give better control than a microbial exhibiting high lethality. It has a better chance to persist in the population and once introduced into the pest population may give long term control for several years (inoculative strategy). A disadvantage can be that this strategy needs more time to bring the pest population down to a non-

damaging level. If immediate effect is needed, e.g. for prevention of defoliation or protection of this year's crop, pathogens exhibiting high lethality have to be used. The appropriate pathogen has to be chosen on a case by case basis.

CONCLUSIONS

Conclusions

At the end of the meeting, the delegates divided into three discussion groups to identify areas where further work was needed and where there were possibilities for future collaboration. The three discussion group topics were:

a) Insect-parasitic nematodes
b) Insect-pathogenic fungi
c) Foliar persistence of insect pathogens

Summaries of these discussions and conclusions are given below:

a) INSECT-PARASITIC NEMATODES

The discussion group was attended by nineteen nematologists with an active interest in biological control and the use of rhabditid nematodes. Seven topics for discussion were on the agenda but two of them were not addressed due to shortage of time. These subjects were: (i) nematode production problems and (ii) evaluation of the effects of natural enemies and pathogens of nematodes, on field applications of rhabditids.

Proposals and Conclusions

1. Nematode taxonomy

Identification of rhabditid nematodes still causes problems. In Europe, there are no specialist taxonomists for the genera Steinernema and Heterorhabditis and, because of the inconvenience of sending specimens to Australia or the USA, some workers have tended not to bother to determine, ~~to species level~~, the nematodes that they have discovered and are studying. To help overcome these problems it was proposed that two participants in the discussion group, Dr.C.LAUMOND and Mr.P.RICHARDSON, would together produce a document outlining standard procedures for the fixation, staining and mounting of various life stages of rhabditids in order to encourage all workers to use the same techniques. LAUMOND and RICHARDSON also volunteered to offer a second opinion as to the identity of dubious isolates.

2. New isolates of Steinernema and Heterorhabditis

It was generally felt that there is a requirement for new isolates of nematodes and that searches in European soils were providing potentially valuable biological control agents. Those strains exhibiting enhanced infectivity at lower soil temperatures were considered to be particularly desirable and useful in European conditions. The meeting made clear its intention to more-freely exchange isolates with the prospect, perhaps, of eventually carrying out comparative tests involving a range of isolates against a common pest.

3. Bioassay techniques

The group was unanimous that standard methods of bioassay were much needed. Dr.K.DESEO agreed to make available details of her filter paper/Galleria method for Steinernema and Dr.P.WESTERMAN would circulate the method used by Dr.SIMONS for Heterorhabditis. These, and any new techniques ~~that may~~ arise, should be tested and the results freely-exchanged.

4. Impact of nematodes on non-target organisms

This topic aroused much interest but no direct action. It was felt, however, that if future field trials could be modified to involve plots that might assess side-effects of nematode applications on non-target organisms then these should be included in the experimental design.

5. Compatibility of nematodes with chemicals

Dr.K.DESEO undertook to provide and circulate an English translation of a paper by Dr.A.KOVACS concerning the effects of a range of insecticides, fungicides, herbicides and growth regulators on the mobility of the Breton strain of Neoaplectana carpocapsae.

P.N.Richardson

b) INSECT-PATHOGENIC FUNGI

The following topics were seen to be important in future research on entomopathogenic fungi:

1. List of Hyphomycetes recorded on curculionid weevils
2. Distribution and natural occurrence of entomopathogenic fungi in soil
3. Bioassay methods
4. Characterisation of strains
5. Mass production: Influence of production techniques on behaviour of fungi in soil
6. Selection of isolates which can infect at low or high temperatures
7. Suppression of fungal activity in soil
8. Development of selective media for reisolation of entomopathogenic fungi from soil
9. Use of genetics to produce 'new strains'

Future collaborative opportunities with greatest priority were believed to be:

1. Distribution and natural occurrence of entomopathogenic fungi in soil

There was a great interest in the use of the Galleria bait method in different countries and areas. Also the use of other bait insects was suggested to look for new fungal species and strains. In addition, it was proposed to test the suitability of selective media. These methods also offer the possibility to isolate and select fungi with different temperature requirements.

Persons concerned: MARCHAL and RIBA/France, COREMANS-PELSENEER/Belgium, GILLESPIE/ United Kingdom, McCOY/USA, LIPA/Poland, ZIMMERMAN/Federal Republic of Germany.

2. Development of selective media for soil-inhabiting entomopathogenic fungi

The work with entomopathogenic fungi in soil implicates the knowledge of their recovery using selective media. ZIMMERMANN agreed to make a compilation of the selective media which have been published and to send this list to persons who are interested (see below - Appendix A).

3. **Bioassay methods**

Some participants were interested in an exchange of ideas and methods of the different bioassays which are used for research on soil insects.

Persons concerned: GILLESPIE/United Kingdom, COREMANS-PELSENEER/Belgium, MARCHAL and RIBA/France.

4. **Abiotic and biotic factors affecting fungal activity in soil**

The papers given at the meeting on the activity and persistence of fungal pathogens in the soil revealed that we need more research on the behaviour of entomopathogenic fungi in different soils and under different conditions.

Persons concerned: RIBA/France, McCOY/USA, ZIMMERMANN/Federal Republic of Germany.

G.Zimmermann

c) FOLIAR PERSISTENCE OF INSECT PATHOGENS

The topics seen as of greatest importance in future studies were:

1. The development of standardised bioassay methods for measuring persistence. Details of assay methods should be exchanged between participants (see Appendix B).

2. Study of pathogen viability in shared formulations.

3. Studies of environmental persistence including physical interactions between the pathogen and plant surface as well as the effects on pathogens of specific regions of the solar radiation spectrum.

C.C.Payne

APPENDIX A

G. ZIMMERMANN

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Compilation of references on selective media for isolation of Beauveria bassiana, B. brongniartii and Metarhizium anisopliae

Beauveria bassiana

BEILHARZ, V.C., PARBERY, D.G. and SWART, H.J., 1982: Dodine: A selective agent for certain soil fungi. Trans. Brit. mycol. Soc. 79, 507-511.

DOBERSKI, J.W. and TRIBE, H.T., 1980: Isolation of entomogenous fungi from elm bark and soil with reference to ecology of **Beauveria bassiana** and **Metarhizium anisopliae**. Trans. Brit. mycol. Soc. 74, 95-100.

KUHLMAN, E.G. and HENDRIX, F.F., Jr., 1962: A selective medium for the isolation of **Fomes annosus**. Phytopathology 52, 1310-1312. (This medium was formerly used by MÜLLER-KÖGLER, but is was never published in detail.)

LINGG, A.J. and DONALDSON, M.D., 1981: Biotic and abiotic factors affecting stability of **Beauveria bassiana** conidia in soil. J. Invert. Pathol. 38, 191-200.

Beauveria brongniartii (= B. tenella)

JOUSSIER, D. et CATROUX, G., 1976: Mise au point d'un milieu de culture pour le dénombrement de **Beauveria tenella** dans les sols. Entomophaga 21, 223-225.

VEEN, K.H. and FERRON, P., 1966: A selective medium for the isolation of **Beauveria tenella** and of **Metarrhizium anisopliae**. J. Invert. Pathol. 8, 268-269.

Metarhizium anisopliae

BEILHARZ, V.C., PARBERY, D.G. and SWART, H.J., 1982: Dodine: A selective agent for certain soil fungi. Trans. Brit. mycol. Soc. 79, 507-511.

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PEREIRA, J.C.R., DHINGRA, O.D. and CHAVES, G.M., 1979: A selective medium for population estimations of **Metarhizium** in soil. Trans. Brit. mycol. Soc. 72, 495.

VEEN, K.H. and FERRON, P., 1966: A selective medium for the isolation of **Beauveria tenella** and of **Metarrhizium anisopliae**. J. Invert. Pathol. 8, 268-269.