Session 7D

Postgraduate Student Posters

Session Organiser	Mr M W Skidmore
Poster Papers	7D-1 to 7D-11

EFFECT OF MODE OF ENTRY ON THE SUSCEPTIBILITY OF THE EGYPTIAN COTTON LEAFWORM TO IMIDACLOPRID

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ABSTRACT

Imidacloprid is five times more toxic and acts more rapidly by oral than by topical application to larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* Boisd. The onset of neurotoxicological symptoms corresponds with the onset of vomiting.

INTRODUCTION

The nitromethylene heterocycle insecticides interfere with cholinergic synaptic transmission in the insect central nervous system (CNS) by binding to the nicotinic acetylcholine post synaptic receptors (Schroeder and Flattum, 1984). Imidacloprid, a chloronicotinyl derivative, has potent systemic activity but its efficacy against certain lepidopteran species is poor (Lagadic *et al.*, 1993). Since sap sucking insects encounter the insecticide via the oral route, and lepidopterans mainly by contact, this study was undertaken to determine whether the observed differences in toxicity are due to the different routes of uptake.

MATERIALS AND METHODS

All larvae were starved for 24h prior to treatment. Imidacloprid was dissolved in acetone/dimethylsulfoxide (60/40), and applied topically (1μl per insect) using a capillary micropipette or orally on a 4mm diameter cabbage leaf disk. Recordings of neurophysiological activity were carried out on single, intact, larvae using the method of Broderick *et al.*, 1991. It was not possible to achieve 50% mortality, at 48h, by topical application to larvae even as small as 25 mg. However, by applying a fixed dose (300 μg per insect) and varying larval weight, a dose response curve was obtained at 72h for both oral and topical application.

RESULTS

Whole animal toxicity and neurotoxicity were estimated for the oral and topical application routes. Thirty larvae were used for each dose level in all whole animal toxicological experiments; 4th instar (50mg) for topical application, and 6th instar (190 and 420 mg) for oral administration. Two symptoms were monitored, vomiting at early times (minutes) and death at 72h. Single larvae were used in the *in vivo* neurotoxicological experiments. There was a marked difference in both mortality and vomiting between the two routes of application. For mortality the compound is 5 times more toxic by oral than by topical application at 72h. For a roughly similar dose (1.97 and 0.51 μg/mg for topical, and 1.53 and 0.72 μg/mg for oral routes) time to onset of vomiting was markedly shorter with oral dosing. All larvae responded following oral administration (ET50 = 3-6 min) but less than 50% responded within 1.5h of topical application (Table 1).

The onset of neurotoxicological symptoms corresponded with the onset of vomiting in poisoned larvae. Neurotoxicity was characterised by an increase in the frequency of action potentials, and a regular pattern of activity which persisted for the duration of the experiment (1.5h) and contrasted with fluctuations in the pre-dosed control period. With a low dose (0.51 μg/mg) by topical application, neurotoxicological symptoms were observed only in those animals which vomited and never in asymptomatic individuals.

Table 1. Dose-response data for vomiting by Egyptian cotton leafworm larvae (30 animals per treatment) following application of imidacloprid.

Route	Topical			Oral		
Larval Weight (mg)	54	51	49	414	422	196
Dose (μg/mg)	5.58	1.97	0.51	0.72	0.05	1.53
Number responding	14	11	13	30	30	30
ET50 (min)	>90	>90	>90	6	5	3

The carrier solvent alone had no toxicological effects.

DISCUSSION AND CONCLUSIONS

Imidacloprid binds to nicotinic receptors which in insects are found only in the CNS (Zwart *et al.*, 1992). Pharmacodynamic activity will not change with route of application within a species, and vomiting corresponds to similar neurotoxicological symptoms for oral and topical application. This indicates that the observed differences are not due to local effects on the gut, but are due to action in the CNS. Accumulation in the CNS will be governed by pharmacokinetic factors. Since insects have a haemocoelic circulatory system, distribution is likely to be similar for both routes of uptake. The rates of penetration may, however, differ for the two routes; this would affect the onset of early symptoms but would be unlikely to affect endpoint toxicity which depends on exposure (μgh) of the site of action (Greenwood *et al.*, 1990). The opportunity for elimination and/or binding as material crosses the cuticle may differ from that when it crosses the gut wall. Work is currently underway to investigate these possibilities.

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CONTROL OF LEATHERJACKETS BY NATURAL ENEMIES: THE POTENTIAL ROLE OF THE GROUND BEETLE *PTEROSTICHUS MELANARIUS*

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ABSTRACT

The ground beetle *Pterostichus melanarius* was found to be able to reduce the numbers of soil-dwelling leatherjackets in the laboratory, possibly by hunting underground using olfactory stimuli. The beetles were more efficient at reducing leatherjacket numbers in the spring than in the autumn and although *P. melanarius* was most active in August, adults that have overwintered emerge to feed in the spring when leatherjacket control would be most effective.

INTRODUCTION

Ground beetles (Coleoptera: Carabidae) have been widely studied as natural enemies of crop pests such as aphids (e.g. Sunderland, 1975), but studies of their predation of larger pests have been mainly limited to surface dwelling caterpillars (e.g. Wallin, 1991). Leatherjackets, the larvae of the cranefly (*Tipula* spp.) attack grasses and cereals above and below the ground. *Pterostichus melanarius*, the most abundant large ground beetle of agricultural land in north-east Scotland, will readily eat leatherjackets in the laboratory. However, in the field, leatherjackets are primarily soil-dwelling, so experiments were carried out to determine whether, and how, *P. melanarius* could prey on leatherjackets under these circumstances.

MATERIALS AND METHODS

Twelve containers (11cm×19cm) were filled with 5cm depth of compost/sand mixture. Four (October '93) or five (May '94) leatherjackets were allowed to burrow into each container. One freshly caught beetle was put into each of seven of the containers in October and six in May, while the remaining containers were controls. Leatherjacket numbers were counted each week until most of them or the beetles had died. To determine whether *P. melanarius* can detect leatherjackets chemically, ten beetles were placed centrally in a tray (50cm×45cm), two quarters of which were covered with clean sand and two with sand in which leatherjackets had been reared. After 30 minutes the positions of the beetles were recorded. This was repeated seven times, each time rotating the tray through 90° to mask any phototactic response by the beetles. The level of activity of *P. melanarius* throughout the summer was monitored by pitfall trapping in grass plots at Tulloch farm, Aberdeen.

RESULTS

The beetles significantly reduced leatherjacket numbers on both occasions, but did so

more quickly and efficiently in May than in October (Table 1). The beetles readily burrowed into the soil and three newly killed leatherjackets that were found were all below the surface of the soil. The beetles seemed to be able to detect the chemical traces of the leatherjackets, with 52 recordings of beetles on the leatherjacket-enriched sand compared with 14 on the clean sand (χ^2=22.7; P<0.001). In the field, *P. melanarius* was most active in August, but with a smaller peak of activity in May (Table 2).

TABLE 1. Mean number of leatherjackets per container. P-values for Mann-Whitney test.

		Start	Week 1	Week 2	Week 3	Beetle v. control
October	With Beetle	4.0	3.7	3.0	2.6	Week 3
	Control	4.0	4.0	4.0	4.0	P<0.05
May (1 week	With Beetle	5.0	0.3	-	-	Week 1
only)	Control	5.0	3.7	-	-	P<0.01

TABLE 2. Numbers of *P. melanarius* caught during one week's pitfall trapping per month (42 traps) in organic grass, 1993.

Month	May	June	July	Aug.	Sept.	Oct.
Number of *P. melanarius*	22	2	43	137	62	1

DISCUSSION

The results show that the number of leatherjackets living in soil can be reduced by *P. melanarius* in excess of mortality from other causes. The recently killed leatherjackets below the soil surface suggest that the beetles may be able to hunt them underground, possibly by following chemical stimuli in the leatherjacket burrows. *P. melanarius* was most abundant in August when craneflies are in the adult stage, but the secondary peak of activity in May coincides with the period when leatherjacket control would be most effective, adding to the high initial mortality that would already have occurred during the winter. The beetles were more efficient at reducing leatherjacket numbers in May than later in the year, probably because they were replenishing depleted fat reserves after the winter.

ACKNOWLEDGEMENTS

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BIOCONTROL OF *SCLEROTINIA SCLEROTIORUM* BY MANIPULATION OF PETAL MICROFLORA

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ABSTRACT

Stem rot of oilseed rape, caused by *Sclerotinia sclerotiorum*, normally infects via ascospores which colonise senescent petals lodged onto lower petioles and stems. Here we demonstrate that, as an alternative to fungicide application, some disease control can be obtained by the application to petals of mixtures of microorganisms. These antagonists were selected using an *in vitro* and *in vivo* screening programme.

INTRODUCTION

Sclerotinia sclerotiorum is an important pathogen of many crops. Oilseed rape (OSR) is the single most important combinable break crop in the U.K. Currently stem rot of OSR is controlled by fungicides applied during the flowering period, designed to prevent infection which normally occurs via fallen petals lodged onto lower stems and leaves. Control is often erratic and uneconomical, mainly because of limited and uneven coverage of the petals by the fungicide. Increased environmental awareness has created a demand for a reduction in the quantity of agrochemicals applied to crops. An effective alternative method is therefore desirable to replace the use of fungicides to control this disease.

Traditional approaches to biological control have selected isolates that show potential in a laboratory screening programme and subsequently tested them individually in small scale field trials. To date, the use of single antagonists has resulted in little commercial success. Biological control occurring naturally in the field is a multifactorial process. The present investigation has attempted to manipulate the microflora of OSR petals to inhibit ascospore colonisation of the infection court.

METHOD

Epiphytic microorganisms (626 isolates) from petals and leaves of OSR, were screened *in vitro* and *in vivo* for biocontrol activity. Mixtures of microorganisms were prepared from the highest scoring isolates. Two mixtures were used: A (reconstituted "natural" saprophytic petal microflora) and B (artificial mixture of the most antagonistic isolates from the screening programme).

Trials were carried out in replicated 14x8m plots of winter OSR cv. Capricorn. Mixture A ($1x10^6$ cells per ml of each organism) was applied (8 litre/100m^2) at weekly intervals during the flowering period. The mixture was applied with or without a nutrient solution in a randomised block design. At the end of the season the proportion of diseased plants was recorded.

The mixtures were compared for their ability to suppress white mould in an *in vivo* detached leaf/ petal bioassay. Defrosted OSR petals (stage A petals; Inglis and Boland, 1990) were co-inoculated with the mixtures and ascospores of the pathogen ($1x10^5$ spores/ml). Petals inoculated only with ascospores served as controls. After 72 hours incubation at 20°C, the diameter of lesions developing on the leaves was recorded.

RESULTS

The two mixtures differed in their constituent isolates. Mixture A contained: a white and pink yeast isolate, *Cladosporium sp.*, *Aureobasidium sp.*, *Penicillium sp.*, a Gram-negative yellow bacterium, fluorescent Pseudomonad, and *Bacillus sp.* Mixture B

contained: *Fusarium sp.*, *Cladosporium sp.*, *Penicillium sp.*, *Mucor sp.*, unidentified *Fusarium*-like fungus and a fluorescent Pseudomonad.

The proportion of plants showing disease symptoms varied significantly between the replicate blocks, because of differences in plant density. The data were analysed as a two factor factorial ANOVA, using the GLIM program with a binomial error structure. The mean proportion of diseased plants ranged from the control, water only (0.68), nutrients only (0.70), mixture A only (0.64); and both mixture A and the nutrient solution (0.48). Changes in deviance associated with removal of any factors or interaction terms from the maximal model were compared with values of χ^2 in tables (table 1).

Table 1. Analysis of deviance table for the mean proportion of diseased plants.

Explanatory variable	Deviance	d.f.	Significance
Nutrient x Mixture A interaction	3.12	1	$0.1 < P < 0.05$
Nutrient	5.93	1	$0.025 < P < 0.01$
Mixture A	7.69	1	$0.01 < P < 0.005$
Block	21.59	5	$P > 0.001$

The interaction term is marginally insignificant (P=0.05), and both factors appear significant; indicating the treatment effects are real. With less variation and block effect, the nutrient x mixture A treatment would have shown a significant reduction in disease compared with the control or the two individual treatments.

Both mixtures inhibited lesion formation on detached leaves in the bioassay. Differences between the mean lesion diameters were separated by the L.S.D. method (table 2).

Table 2. Mean lesion diameter in an *in vivo* detached leaf/ petal bioassay.

Treatment	Mean lesion diameter (mm)
Control	12.0
Mixture A	8.8
Mixture B	2.8
L.S.D. (P=0.05)	3.2

DISCUSSION

The microflora of bean petals has been shown previously to effect the development of white mould of bean, on seedlings in the glasshouse (Inglis and Boland, 1990). The results presented here have demonstrated the potential to manipulate the microflora of the petals, in the field, to reduce the development of stem rot of OSR. The data also demonstrate, in the laboratory, that an artificial microflora made up of highly antagonistic microorganisms can potentially be more effective in suppressing white mould than the natural petal microflora. Field trials are in progress to test this hypothesis.

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DEVELOPMENT OF IMMUNOLABELLING TOOLS TO STUDY INTERACTIONS BETWEEN MICRO-ORGANISMS IN THE BIOCONTROL OF SEED-BORNE DISEASES OF LINSEED

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ABSTRACT

Ultrastructural studies of *Botrytis cinerea*, a seed-borne pathogen of linseed, have shown that it is restricted to the testa of the seed. Immunogold labelling using a monoclonal antibody raised against *Botrytis cinerea* has allowed the identification and location of this pathogen in resin-embedded seed sections.

INTRODUCTION

Botrytis cinerea is an important seed-borne pathogen of linseed in the UK. Evidence suggests that it is restricted to the outermost layer of the testa (Mercer & Hardwick, 1991) possibly in the form of resting hyphae that may resume activity upon imbibition and lead to infection of the emerging radicle. Microscopical studies of thin sections of resin-embedded seed-coats have allowed the detection of fungal hyphae; however such methods have been unable to distinguish *B. cinerea* from other seed-borne pathogens, saprophytes and potential biocontrol agents (BCAs). Recently, some workers have raised monoclonal antibodies (Mabs) against *B. cinerea* to detect conidia and mycelium harvested from other host plants, using indirect immunofluorescence (IF) labelling (Bossi & Dewey, 1992; Salinas & Schots, 1994). In this paper we report the development of an immunogold (IG) technique to detect *B. cinerea* in seed sections of linseed.

MATERIALS AND METHODS

Seeds from a batch known to be predominantly infected with *B. cinerea* were cut longitudinally in half and one half was plated out onto 2% malt agar and incubated according to established methods (Muskett & Malone, 1941). If the pathogen was detected, the testa was excised from the corresponding unplated half seed and retained for further studies. Tissue pieces (3-5 mm^2) were fixed overnight in a mixture of 2% paraformaldehyde and 0.5% glutaraldehyde in 0.2M phosphate buffer pH7 at 4°C. Samples were then dehydrated using a graded acetone series followed by infiltration and embedding in Unicryl resin (British Biocell); which was polymerised with a u.v. source at 4°C for 3 days. Sections 200-250μm thick were cut using an ultramicrotome and mounted on 600 mesh nickel grids.

A hybridoma supernatant from the cell line KH4 supplied by F. M. Dewey raised against an isolate of *B. cinerea* from grapes (Bossi & Dewey, 1992) was used for these studies. A Mab raised against *Phytophthora infestans* in the department was used as a control. Sections were blocked with 10% rabbit serum in PBS (pH 7.2) for 15 min, the excess decanted, and then incubated for 30 min at 37°C with KH4 hybridoma supernatant diluted 1:5 in PBS. Sections were then washed with PBS, incubated with biotinylated rabbit anti-mouse antibody (Zymed) for 10 min and then further washed with PBS. Sections for IG labelling were incubated with 10ηm diameter streptavidin-gold conjugate (British Biocell) diluted 1:30 in 1% bovine serum albumin (BSA) for 30 min, and washed sequentially in 1% BSA, PBS and finally water. Sections were then silver-enhanced (British Biocell) for 7 min, washed with water, counter-stained with uranyl acetate, further washed with water and examined with a transmission electron microscope at an accelerating voltage of 100kv.

RESULTS & DISCUSSION

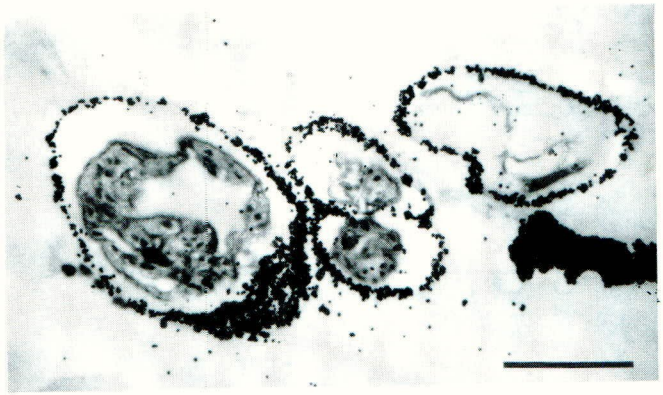

Fig. 1. Electron-dense gold particles on walls of *B. cinerea* hyphae in a section of the outer most layer of the seed coat. bar = 4 μm.

Electron-dense gold particles were observed on the walls of hyphae of *B. cinerea* in sections previously treated with the KH4 Mab (Fig. 1) but not when the *P. infestans* Mab was used. The specificity of the KH4 Mab was tested, using IF, against the other commonly occurring seed-borne pathogens and saprophytes such as *Alternaria linicola*, *A. alternata*, *Stemphylium botryosum* and *Epicoccum nigrum*. It was found that *A. linicola* did show fluorescence labelling with the KH4 Mab both in resin-embedded seed-sections and pure culture. However, this cross reactivity could be suppressed by titrating the Mab against the level of the fluorescent signal observed through a fluorescent light microscope. A 1/30 dilution of the KH4 hybridoma supernatant with PBS was found to subdue sufficiently the non-specific labelling of *A. linicola* to allow the distinction between it and *B. cinerea* to be made in seed-sections.

The immunolabelling technique developed will be used to study the mechanisms of biocontrol of another seed-borne pathogen, *A. linicola*. Earlier *in vitro* dual culture tests with *A. alternata* and *E. nigrum* have demonstrated good efficacy in the biocontrol of this pathogen. Mabs are being developed against *A. linicola* and *A. alternata*. This would then allow the immunolabelling of these organisms using the technique developed in this study.

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POTENCY AND ULTRASTRUCTURAL EFFECTS OF TEBUFENOZIDE ON *SPODOPTERA EXIGUA*

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ABSTRACT

This study indicates that the hydrazine-based ecdysteroid agonist tebufenozide possesses a high insecticidal activity on third-instar larvae of the beet armyworm, *Spodoptera exigua*, by ingestion of treated diet, topical application and residual contact. The phenotypic and ultrastructural changes were studied by transmission electron microscopy. Treated larvae showed characteristic moulting effects upon treatment: clear signs of premature apolysis were noted and 24 h after treatment a double abnormal cuticle was visible.These observations strengthen the moulting accelerating mode of action of tebufenozide and its inhibitory activity on post-apolysis processes in susceptible species. Further details and fine cuticle structure are discussed.

INTRODUCTION

Tebufenozide is a novel synthetic nonsteroidal ecdysteroid agonist which represents a new class of insect growth regulators and which induces, especially in larval Lepidoptera, a premature and lethal moult by direct stimulation of the ecdysteroid receptors (Wing, 1988; Wing *et al.*, 1988; Silhacek *et al.*, 1990; Heller *et al.*, 1992; Smagghe & Degheele, 1994).

The present study evaluates the insecticidal potency of tebufenozide to kill third-instar larvae of the beet armyworm, *Spodoptera exigua* (Hübner), (Lepidoptera: Noctuidae) a worldwide pest in vegetables and ornamentals. In addition, we aim to provide evidence for the novel mode of action of this compound by studying its effects on the cuticle deposition and ultrastructure of third instars.

MATERIAL AND METHODS

Insects

S. exigua-larvae were routinely reared on a modified Poitout-diet with added ground alfalfa and at 25±2°C, 75±5% r.h. and a 16:8 (L:D) photoperiod.

Compound and treatments

Tebufenozide (24% SC) was kindly supplied by Rohm and Haas Co.

For ingestion experiments, semi-artificial diet to which different quantities of tebufenozide were added, was continuously supplied. Topical treatment was performed by dorsal application of 0.5 µl/larva of different acetonic dilutions of the compound as previously described (Viñuela, 1982). In the residual contact assay, different solutions of the compound were prepared in distilled water and 1 ml sprayed on glass plates using a potter spray tower, as previously reported in detail (Jacas *et al.*, 1992). In all series, mortality percentages included both death and affected individuals and were subjected to probit analysis using POLO-PC (LeOra Software, 1987).

For electron microscopy, control and treated larvae were collected at regular intervals and generally prepared following the techniques of Hayat (1986).

RESULTS AND DISCUSSION

Insecticidal activity on third-instar larvae of *S. exigua*

Biological activity assays indicated that at biologically high active doses tebufenozide induces a premature, abnormal and lethal moult in third-instars within 24 h. The head capsule slipped down, revealing a double head capsule; moreover, underneath the old cuticle a fragile and often nonsclerotized new head capsule was oberved. Likewise, larval feeding and weight gain were significantly suppressed, and in some cases loss of haemolymph and an extrusion of the hindgut were seen. Most of such larvae died in their old cuticle, however, in some cases the old cuticle was partially shed with remnants remaining on the new cuticle. In such treatments, normal pupal formation only reached (very) low percentages and adult emergence nearly never occurred. Fifty percent of third-instar larvae were killed by 1.937 µg AI/g diet, 0.414 µg AI/larva and 358.8 µg AI/ml by ingestion of treated diet, topical application and in a residual contact assay, respectively, indicating tebufenozide possesses a promising insecticidal activity.

Effects on the cuticle deposition and ultrastructure in third-instar larvae of *S. exigua*

Electron microscopic observations revealed that in treatments apolysis was prematurely induced leading to double cuticle formation. Shortly after treatment, putative ecdysial droplets appeared, the endoplasmatic reticulum increased drastically and a hypertrophy of the Golgi complexes was observed. Further on, an ecdysial space was prematurely formed and a new larval cuticle was deposited which reveals a sequential change similar to a normal moulting process. However, a normal cuticle structure was interrupted since the endocuticular lamellae were conspicuously absent and resorption of the ecdysial droplets seemed to be inhibited. Likewise, epidermal cells showed signs of hyperactivation and degeneration. Thus, observations refer to a hyperecdysteroid action and strengthen the moulting accelerating activity of this compound and its inhibitory activity on post-apolysis processes.

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THE EFFECTS OF BEET WESTERN YELLOWS VIRUS ON THE GROWTH AND YIELD OF WINTER OILSEED RAPE

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ABSTRACT

Field plots of winter oilseed rape were artificially inoculated with beet western yellows virus in the autumn. The virus had no effect on the growth of the crop during the early development stages. However it decreased height at flowering and pod development, and decreased dry weight at pod development. Final seed yields were decreased by 26%.

INTRODUCTION

Beet western yellows virus (BWYV) is a phloem specific luteovirus which is transmitted by the aphid *Myzus persicae.* BWYV infects a number of economically important crops, including winter oilseed rape. Smith and Hinckes, 1984, have shown that the oil yield of heavily infected rape plots was 13% lower than that of lightly infected plots, however subsequent trials have proved inconclusive (Hill, Lane and Hardwick, 1989). This trial aims to determine the effect of the virus on the growth of the crop by using artificial inoculation rather than relying on natural infection.

MATERIALS AND METHODS

A trial was carried out on a commercial crop of winter oilseed rape (cv. Capricorn) at Wickhambrook, Suffolk during 1993-1994. At growth stage 1.5 - 1.6 (Sylvester-Bradley and Makepeace, 1984) 8 plots, 6 m x 22 m, were artificially infected with BWYV using viruliferous aphids which had been cultured in a glasshouse. All plots, including untreated, were sprayed with pirimicarb at 140 g AI/ha six days after inoculation. The dry weight (all plants) and height (20 plants) from 1 m^2 samples from infected and non-infected plots were measured at intervals during the growth of the crop. On each occasion twenty plants per sample were tested for BWYV using enzyme linked immunosorbent assay (ELISA) to determine the percentage of infected plants per plot. Plots were harvested by combine on 4 August 1994, to determine final yield.

RESULTS AND DISCUSSION

Control and inoculated plots had 10% and 90% infection levels respectively. There were no significant differences between infected and healthy plots at early

growth stages. However, after stem extension, the height of infected plants was 12% less than healthy plants in April at 50% flowering, 7% less in May at early pod development, and 14% less in June at mid-pod development (Table 1). There was also a 12-23% decrease in dry weight of infected plants in May and June, but not in July. Final seed yields of 4.14 t/ha and 3.04 t/ha were obtained for control and infected plots respectively adjusted to 91% dry matter (SED 49.02), showing a decrease of 26% due to infection.

TABLE 1. The effects of BWYV on the weight and height of oilseed rape

Sample date	Mean dry wt g/m^2		SED	Mean ht (cm)		SED
	Control	Infected		Control	Infected	
8.11.93	90.8	86.1	8.14			
7.12.93	94.2	101.8	8.28			
8.2.94	120.9	115.6	11.89			
31.3.94	236.0	216.1	17.17			
27.4.94	489.0	442.6	29.11	77.8	68.5**	2.84
23.5.94	732.7	642.4*	39.70	113.6	105.8*	3.08
20.6.94	1049.1	810.8**	75.59	118.5	102.2***	3.29
20.7.94	1064.8	1020.8	59.14			

*, **, *** = significant difference from control at p = 0.05, 0.01 & 0.001 respectively.

This study shows that BWYV affects both the growth and the yield of oilseed rape. The incidence of BWYV can be decreased by targeting the aphid vectors. Pyrethroid insecticides used for flea beetle control in the early autumn will control aphids and also decrease virus levels.

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THE BIODIVERSITY OF COLEOPTERA OVERWINTERING IN ARABLE FIELD BOUNDARIES

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ABSTRACT

Coleoptera populations were assessed from the boundaries surrounding a single field. There were considerable differences between boundaries in physical and vegetational structure and in their ability to support a diverse coleopteran fauna. The maintenance of between boundary variation is important for the preservation and enhancement of farmland biodiversity.

INTRODUCTION

'Biodiversity' can be defined at the species level in terms of genetic variation and at the habitat level in terms of numbers of habitats and the numbers of species within them (Altieri, 1991). Many believe that the more interactions between species the greater is the stability of an ecosystem (Pimm, 1984). In arable crops there is a negative relationship between aphid pest levels and arthropod diversity (Potts and Vickerman, 1974) which would seem to support the hypothesis concerning complexity and stability; despite this, most research into arthropod ecology in arable ecosystems has concentrated on individual species and biodiversity is rarely considered.

This investigation examined the Coleoptera, overwintering in field boundaries. Biodiversity, quantified in terms of the number species (richness) and the number of individuals (abundance), was assessed in the boundaries of one field. One of the boundaries was a 'beetle bank': a ridge sown with tussock-forming grass, which supports large numbers of beneficial arthropods over winter (Thomas *et al.*, 1991). The other boundaries ranged from a simple grass strip to a woody hedge bordered with a variety of vegetation. The differences between the coleopteran species found in each boundary, the ability of each boundary to support a diverse coleopteran fauna and variation within each boundary was assessed. The findings will help answer questions about how we best manage field boundaries to maximise farmland biodiversity.

MATERIALS AND METHODS

The work was carried out on a 13 ha field on the Manydown Estate, N. Hampshire. Five boundaries were sampled: a four year old beetle bank (boundary 1); a narrow grass strip bordering a farm track (boundary 2); a grass ridge bordering the same track (boundary 3); a grass strip bordering a copse (boundary 4) and a raised bank of mainly broad-leaved vegetation beneath a woody hedge (boundary 5).

Twenty samples were taken from each boundary. Sampling consisted of digging up turves of 0.04m^2 to a depth of 10cm. The samples were sorted by hand in trays.

RESULTS

128 species (13 families) were found in all samples. The mean number of species per sample ranged from 14.7 in boundary 1 to 5.5 in boundary 2. Only 1 of these species was found in boundary 2 alone. In contrast 20 species were found exclusively in boundary 5. The total number of species found in each boundary was 69, 39, 59, 64 and 77 for boundaries 1-5 respectively. One-way analysis of variance (ANOVA) followed by Tukey's test gave no significant differences, at the 95% level, between the number of species found in boundaries 1 and 5, 5 and 4 and 4 and 3. Boundary 2 had significantly fewer species than the other boundaries. The variance:mean ratios of species numbers, measures of within-boundary uniformity, showed boundary 1 to be the most uniform habitat and boundary 5 the least.

Of the 2756 individuals found in all the turf samples 1026, 166, 440, 452 and 672 were found in boundaries 1 to 5 respectively. One way ANOVA showed boundary 1 to contain significantly more and boundary 2 significantly fewer individuals than all other boundaries.

DISCUSSION

The beetle bank was a uniformly suitable habitat for many species but failed to support the diversity of the more structurally complex boundary 5. The relationships between boundary structure and biodiversity will be reported elsewhere. The results point to the need to maintain a wide range of boundary types to maximise coleopteran biodiversity. The findings of species unique to single boundaries may be an effect which would be nullified by an increase in sample size. However, boundary diversity may lead directly to increased biodiversity even on an individual farm scale.

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FACTORS AFFECTING THE CONTROL OF *OTIORHYNCHUS SULCATUS* WITH ENTOMOPATHOGENIC NEMATODES, IN STRAWBERRIES GROWN ON RAISED BEDS

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ABSTRACT

The temperature profile relating to the efficacy for the UK isolate of *S. carpocapsae* against the black vine weevil, *O. sulcatus*, was clearly delineated between 15 and 33°C. The nematode can be delivered through a drip irrigation system without loss of viability and the distribution of the nematodes through two T-tapes along and across the raised beds was very satisfactory. Nematodes can be applied either during the late summer or early autumn or in the late spring when temperatures are high enough to give satisfactory control.

INTRODUCTION

The black vine weevil, *Otiorhynchus sulcatus* (BVW) is a major pest of strawberries, particularly those grown on raised beds. Entomopathogenic nematodes are now being used to control BVW (following the withdrawal of the persistent organochlorines). The present experiments were undertaken to investigate the distribution of nematodes in drip irrigation and the relationship between temperature and efficacy.

MATERIALS AND METHODS

Two hundred infective juveniles of *Steinernema carpocapsae* (IJs) were pipetted evenly onto moist, washed and autoclaved sand in 35 x 15 cm petri-dishes. One late instar larva of *O. sulcatus* was placed on the sand, the dishes sealed with Parafilm and placed on a temperature gradient plate (Murdoch *et al.*, 1989). The larvae were exposed to temperatures in the range 8 - 35°C for 6 days.

Strawberries grown on raised beds are normally irrigated and fertilised through single lines of T-tape but nematode distribution was poor (Kakouli, 1994) and therefore in the present experiments two lines of T-tape were used (Fig. 1). In the summer/autumn of 1993, 40 mature eggs of the BVW were introduced onto 4 plants at 17 m, 47 m and 77 m from the top of a 94 m bed. The dosage of *S. carpocapsae* was 2 billion nematodes per treated hectare, injected into the irrigation system 100 m from the field. For the spring 1994 experiment, five 3/4 instar larvae of BVW were inoculated into soil around 4 plants at the same distance from the top of the row as in the first experiment. To estimate the distribution of nematodes along and across the bed soil samples were taken at 17 m, 47 m and 77 m along the bed and at the points S1, S2, S3 and S4 across the bed: the soil was baited with greater wax moth (Fan and Hominick, 1991). The number of living larvae of BVW were assessed at 4 weeks (1993) and 2 weeks (1994) after application of nematodes.

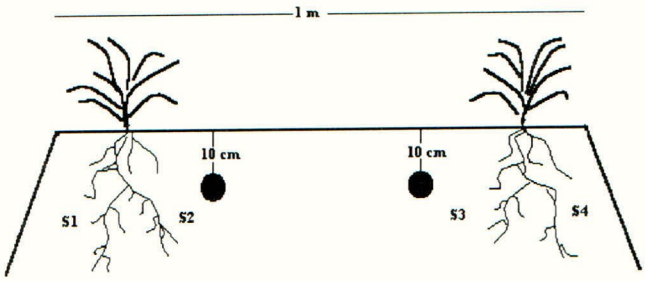

Figure 1 The profile for strawberries grown on a raised bed with two T-tapes. S1, S2, S3 and S4 are the sampling points across the bed.

RESULTS AND DISCUSSION

The temperature profile for the UK isolate of *S. carpocapsae* indicates that this nematode is ineffective below 15°C. IJs will invade at 10°C, but hosts are not killed unless the environmental temperature exceeds 15°C.

The distribution of nematodes across and along in 1993 was very satisfactory (Table 1) and results in 1994 were similar: the level of control was 50% in the 1993 trial and 65% in the 1994 trial. Control of BVW with *S. carpocapsae* should be limited to the late summmer/early autumn or late spring when temperatures are close to 15°C or higher. Kakouli (1994) has shown that all stages of BVW are susceptible to *S. carpocapsae,* but to minimize damage to the crop, nematode treatments should be targeted at the small larvae found in the late summmer/early autumn. Annual or bi-annual treatments with nematodes should keep the BVW population below the threshold at which damage to the crop occurs.

Table 1 The mean number of nematodes recovered from 250 ml soil samples across and along the bed. A 17 m, B 47 m and C 77 m along the bed.

	S_1	S_2	S_3	S_4
A	25	48	50	46
B	18	21	62	41
C	12	23	20	12

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AN ANALYTICAL APPROACH TOWARDS THE DESIGN OF PESTICIDE REGULATION

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ABSTRACT

Debate has often been associated with Directives aimed at regulating pesticides in the European Union (EU). Consistency and flexibility in pesticide policy have been identified as critical themes in the design and achievement of regulatory objectives. Possible strategies for an analytical approach towards the design of pesticide regulation are presented and their implications discussed.

INTRODUCTION

Regulations concerning the environmental fate and effects of pesticides have historically been the responsibility of many different levels of legislative authority. In addition the principles by which pesticide policy is established are inconsistent. Such variations have often arisen because there has been no ordered foundation upon which regulatory design is based (Weale, 1992). The European Directive 80/778/EEC, relating to the quality of water intended for human consumption (European Commission, 1980) provides an example of legislative inconsistency. Under parameter 55, 'pesticides and related products', a uniform standard of 0.1µg/l for the maximum admissable concentration (MAC) of a single pesticide in drinking water is prescribed. Inconsistency arises where other substances controlled under the Directive are subject to varying MAC's according to their individual properties. The Directive has been subject to debate with regards to the scientific rationale upon which its standards were based. An analytical approach towards the design of regulation can play a valuable role in the development of a coherent policy system. In order to build 'optimum' regulatory frameworks emphasis should be placed on matching appropriate solutions to problems, it is therefore, important that problem identification and analysis is undertaken (La Gra, 1990).

APPROACHES TOWARDS REGULATORY DESIGN

Clear definition of a problem, its key processes and components, provides the basis for the targeting of relevant solutions to that problem (Norton and Mumford, 1993). One such approach involves the use of problem trees, providing a procedure to aid the visualisation of cause and effect relationships in a particular situation (La Gra, 1990). Refinement of these models can aid the identification of groups of pesticide problems which lend themselves to particular types of policy actions.

Overuse and misuse of pesticides can increase the negative effects of agrochemical use such as environmental contamination, pest resistance, costs to growers and consumers and possibly effect human health. The causes of this 'core problem' can be attributed to a variety of sources including: the use of inappropriate pesticides and the use of inappropriate application and timing techniques due to a lack of farmer education and training.

Problem analysis allows policy frameworks to be developed which address issues, both at the consequential level such as the monitoring and setting of environmental quality standards, and at the source level, for example, farmer information and manipulative approaches such as taxes and subsidies. An example of such a policy framework is the pesticide risk reduction programme which has progressed in Sweden since the mid 1980's. A 50% reduction in pesticide active ingredient usage has been achieved through a comprehensive framework of new

legislation requiring training and education of agricultural sprayers, taxes on pesticides to finance extension and research and increased food and drinking water residue monitoring (Anon, 1992). Similar policies towards the structured decrease in the use of pesticides are also being undertaken in other European countries such as Denmark and the Netherlands.

A policy framework based on the examination of the causes and effects of the issues to which regulation is directed can lead, in part to a more consistent regulatory system. The targeting of appropriate policy instruments to particular cause and effect relationships is important in promoting improved policy implementation. This dual approach recognises the integral nature of pesticide regulation and therefore, to some extent combats the fragmented approaches typical of such control arrangements in the past.

Greater consistency can be achieved by developing policy frameworks through a stringent combination of science, expert judgement and the recognition of public opinion, provided that the criteria upon which regulatory requirements are established are explicitly recognised and justified.

Within the constraints imposed on regulatory design arising from the need for consistency a policy framework should also recognise the need for a degree of flexibility. Unnecessary rigidity fails to acknowledge the complexity of pesticide control arrangements as well as the diversity of Member States in the EU subject to centrally driven legislation. The incorporation of responsive and adaptable mechanisms into regulation allowing, for example, for advances in technology and risk assessment must also been seen as fundamental to the design of a coherent pesticide policy system.

CONCLUSIONS

Due to expanding demands on the pesticide regulatory system in the EU a coherent and consistent approach towards regulatory design has become increasingly important. Analytical approaches towards the design and implementation of regulations can play an important role in the structure of coherent policies. Clear targeting of appropriate policy actions to particular issues, while maintaining an optimum level of regulatory adaptability is a key element in the realisation of policy objectives.

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OVERWINTERING AND EARLY DISEASE DEVELOPMENT OF *RHODODENDRON* POWDERY MILDEW

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ABSTRACT

Rhododendron powdery mildew is currently causing widespread damage to numerous *Rhododendron* bushes. The development and overwintering of this disease was studied and practices are suggested which may help control or decrease epidemics in the future.

INTRODUCTION AND AIMS

Rhododendron powdery mildew is a fungal disease that was absent in this country fifteen years ago. Current attempts to control the disease are proving costly (Fisher, 1991). The studies reported in this paper aimed to study the method of overwintering of this pathogen and to monitor the epidemiological data in order that a managed disease control programme could be developed.

MATERIALS AND METHODS

Field observations were made on four susceptible *Rhododendron* bushes to investigate the method of overwintering of the powdery mildew. These were as follows :- A -*Rhododendron* Letty Edwards, B-*Rhododendron* Lady Bessborough, C-Unknown hybrid, D-*Rhododendron* Letty Edwards.

Fresh *Rhododendron* leaf samples from each bush were observed regularly throughout the winter (1993 - 1994) using a scanning electron microscope (SEM), (CAMScan microscopes Ltd.) to investigate whether the fungus overwintered on the foliage of *Rhododendron* bushes.

In addition approximately five hundred newly emerged leaves on each bush were monitored regularly from May 1994 for the first signs of disease symptoms (yellow chlorotic spots with purple margin) and to investigate any difference in the onset of an epidemic between *Rhododendron* bushes A - D.

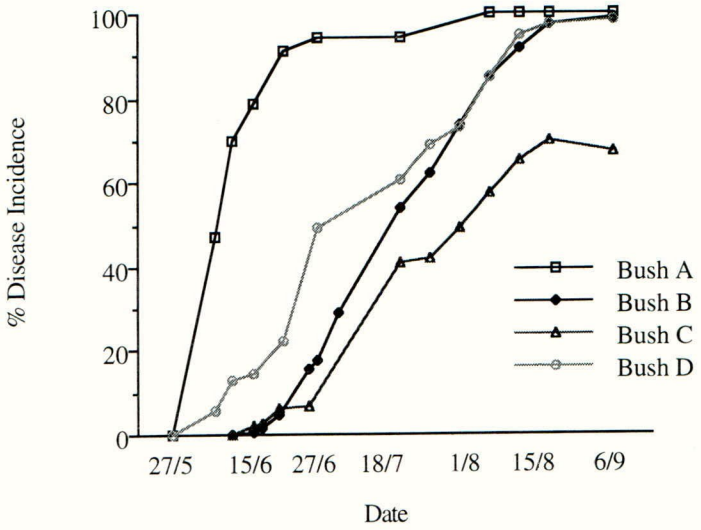
Investigation into the effects of humidity on spore germination were also studied in the field using environmental monitoring equipment (Grant Squirrel data logger) and *in vitro* spore germination experiments were carried out by observing 500 spores at 100% and 0% relative humidity at constant 19 ^{o}C.

RESULTS

SEM observations of lesions on *Rhododendron* leaves that had overwintered indicated large areas of dead hyphae over the infected area. The fungus had been killed either due to the host response to the disease e.g. *Rhododendron* bush C, or alternatively due to environmental conditions e.g. Bush B (more exposed than bushes A, C or D). Bushes A and D showed solitary viable hyphae amongst dead mycelium. Spores that had germinated and produced appresoria were also occasionally observed.

Epidemics of powdery mildew occured on all four bushes, but the epidemics started on the 27/5/94 on bush A and D. Disease incidence data indicated that *Rhododendron* bush A and D had 47.7% and 5.7% infection respectively on the 6/6/94. The epidemics on bushes B and C started on the 15/6/94. This indicates that initial inoculum is derived from the overwintered fungus on bushes A and D.

Figure 1. Onset of disease and percentage newly emerged leaves infected over time on different bushes.



High humidity was shown to be important for the germination of *Rhododendron* powdery mildew spores as *in vitro* results indicated after 24h incubation 62% germination at 100% humidity compared to 17% at 0% humidity.
Earlier field data indicated a 1.8 fold increase in disease rate on bushes where the humidity was significantly greater over a 6 month period (Beales, 1993).

CONCLUSIONS

The powdery mildew was observed to overwinter on the foliage of *Rhododendron* bushes in the form of solitary hyphal elements or occasionally as conidia.
The fungus may have survived the winter on bushes A and D due to a warmer microclimate on leaf surfaces, compared to the surrounding air temperature as these bushes were more sheltered than bushes B and C. In addition *Rhododendron* leaves respond to low atmospheric temperatures by curling (Levit, 1980, Nilson, 1987) therefore the temperature and humidity in the leaf will be greater than the surrounding environmental conditions.
The inoculum for epidemics appeared to be derived from bushes where the fungus overwintered (Bush A and D). Therefore the following practices may help control / decrease future years epidemics, a) Spray during the winter, b) increase exposure of bushes to winter conditions, c) increase spacing of plants thereby reducing humidity.

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SPATIAL AND TEMPORAL CHANGES IN APHID/PARASITOID DISTRIBUTIONS FOLLOWING AN INSECTICIDE APPLICATION TO WINTER WHEAT

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ABSTRACT

The densities and distributions of cereal aphid, primary and hyper-parasitoid populations were monitored in a winter wheat crop before and after a pyrethroid insecticide application at two dose rates. Aphid and parasitoid populations were significantly reduced, with differing patterns of reinvasion and recovery of populations occurring over time in 4 ha plots. The importance of scale and the spatial arrangement of hosts to the evaluation of pesticide side effects on parasitoids is briefly discussed.

INTRODUCTION

The spatial distribution patterns of insect hosts in the field has rarely been documented and the corresponding distributions of their associated hymenopteran primary- and hyper-parasitoid populations is even less well known. An understanding of these spatial relationships is important in designing and interpreting integrated pest management programmes and also in assessing the short and long-term impacts of agrochemical applications.

In this study, the effects of a pyrethroid insecticide, deltamethrin, on the biocontrol ability of the surviving and/or re-invading parasitoid populations in a winter wheat crop were investigated at two dose rates. At a time when dose reductions are employed more frequently, it is important to understand the implications for the efficiency of parasitism.

MATERIALS AND METHODS

The experiment was carried out in the summer of 1993 in a winter wheat crop in Hampshire. The experimental plots each measured 200m x 200m (4ha) and were arranged randomly in the field. Two treatments of insecticide were applied: the full recommended field rate of deltamethrin (Decis, 2.5% EC), and a reduced dose rate representing 1/20th of the field rate. A control plot was left unsprayed.

The sampling regime employed a combination of visual aphid counts, D-vac suction

sampling and sticky trap catches to cover the whole area of the plots, up to 36 days after treatment. A grid arrangement was employed to permit mapping procedures to be undertaken.
Analysis of the data initially followed the methods employed by Duffield & Aebischer (1994) to describe the changes in insect population numbers at a given position in a treatment plot, by taking into account the pre-treatment numbers in that plot and the population changes in the untreated plot.

RESULTS AND DISCUSSION

In the 1/20th field rate sprayed plot, the percentage reduction in numbers of *Sitobion avenae*, total primary-parasitoids and total hyper-parasitoids were 40%, 60% and 54% respectively, whereas in the field rate sprayed plot they were 78%, 90% and 47% respectively.
Reinvasion and recovery trends were influenced by the highly dispersive nature of the aphid parasitoids, with reinvasion into the centre of treated plots occurring relatively quickly (within days after treatment). Overall parasitism levels were relatively low throughout the season, so the true effect of the insecticide applications upon the foraging ability of parasitoids cannot be determined.
The cereal aphid populations were slow to increase in the field rate sprayed plot with eventual "hot spots" or small aphid outbreaks occurring in distinct areas. Within the 1/20th field rate sprayed plot the spatial distribution of the aphids was altered giving a lower density but more homogenous distribution over the plot, and with recovery to control levels occurring rapidly.

Further detailed analysis of the data set will explore the more intricate relationships between the reinvasion and aggregation of the aphid and hymenopteran populations, providing insights into the direct and indirect impacts caused by insecticide applications.

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