POSTER SESSION 4D

POST-GRADUATE STUDENT POSTERS

Session Organiser	Professor P E Russell *AgrEvo UK Ltd, Saffron Walden, UK*
Poster Papers	4D - 1 to 4D - 9

Development of a PCR based diagnostic technique for light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape

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ABSTRACT

Using sequence information from a region flanking the mating type genes of the light leaf spot pathogen (*Pyrenopeziza brassicae*) diagnostic polymerase chain reaction (PCR) primers were designed which reliably distinguished between *P. brassicae* and other fungi pathogenic on winter oilseed rape. A set of primers was also designed which distinguished between the two mating types of *P. brassicae in vitro*.

INTRODUCTION

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae* (anamorph *Cylindrosporium concentricum*), is a major disease of winter oilseed rape (*Brassica napus* subsp. *oleifera*) in the UK, causing losses estimated at >£30 M per annum (Fitt *et al.*, 1997). Although epidemics are often initiated in the autumn (which is the optimal time for fungicide treatment), disease symptoms are invariably not visible until the following February or March. Consequently, fungicide spray decisions are often taken without knowing if crops are affected by the disease. As part of a disease forecasting scheme being developed to improve the rational application of fungicides to control light leaf spot (Fitt *et al.*, 1996), a polymerase chain reaction (PCR) based diagnostic technique is being developed to identify infected plants early in the season, prior to symptom development.

MATERIALS AND METHODS

DNA was extracted from single spore isolates of *P. brassicae* of both mating types (MAT 1-1 and MAT 1-2) according to the method of Raeder & Broda (1985). Mating type specific PCR primers were designed using the sequence of a PCR product closely linked to the mating type loci of both mating types (MAT 1-1 and MAT 1-2) of *P. brassicae* (Ashby, 1997). The sequence of this fragment is almost identical for the two mating types with the exception that the MAT 1-1 sequence contains an extra 95 base pairs (bp) which represents an incomplete repeat of the sequence preceding it, this repeat is absent in the MAT 1-2 sequence. Thus a single primer pair can be used to differentiate between the mating types on the basis of the size of the PCR amplification product generated. The *P. brassicae* specific primers were designed from sequence flanking the mating type loci (G. Singh, pers. comm.). All PCRs were done according to standard protocols.

RESULTS AND DISCUSSION

Amplification using the mating type specific primers produced a 440 bp fragment from all isolates of mating type MAT 1-2 that were tested. A 535 bp fragment was amplified from MAT 1-1 isolates; however this was not amplified consistently and frequent amplification failures occurred. This may have been due to either variability which may be present within MAT 1-1 isolates at the 5' priming site or the presence of secondary structures formed within the extra 95 bp present in MAT 1-1 isolates, as loop forming sequences have been identified within this region. However, this primer pair can be used to determine the mating type of an isolate of *P. brassicae* using pure culture DNA as starting material. This is a substantially quicker method than the traditional method of crossing isolates with others of known mating type, a procedure which can take up to five weeks before fertile ascocarps are produced.

The primer pair Pb1/Pb2 were designed from sequence downstream from the mating type genes; this sequence was fully homologous between the two mating types. Use of these primers in PCRs generated a 750 bp amplification product from each of 50 *P. brassicae* isolates tested. This product was not amplified from other fungal pathogens of oilseed rape including *Leptosphaeria maculans*, *Alternaria brassicae*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum* and *Verticillium dahliae*, or from other fungi such as *Nectria haematococca*, *Neurospora crassa* and the closely related *Tapesia yallundae*. Using these primers, a 750 bp product was amplified from DNA extracted from infected *B. napus* leaves but not from uninfected leaves. Further work is currently being done to determine the earliest point in the infection process at which this technique can detect infection by *P. brassicae* and whether this technique can be successfully combined with field sampling to identify crops at risk from light leaf spot epidemics.

ACKNOWLEDGEMENTS

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REFERENCES

Ashby A M (1997). A molecular view through the looking glass: the *Pyrenopeziza brassicae-Brassica* interaction. *Advances in Botanical Research* **24**, 31-70.

Fitt B D L; Gladders P; Turner J A; Sutherland K G; Welham S J (1996). Predicting risk of severe light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape in the UK. *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases*, 239-244.

Fitt B D L; Gladders P, Turner J A; Sutherland K G; Welham S J; Davies J M (1997). Prospects for developing a forecasting scheme to optimize use of fungicides for disease control on winter oilseed rape in the UK. *Aspects of Applied Biology* **48**, 135-142.

Raeder U; Broda P (1985). Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**, 17-20.

Effects of fluquinconazole seed treatment on the cereal take-all fungus and antagonistic rhizosphere and stem-base fungi

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ABSTRACT

Rhizosphere and stem-base fungi from crops of wheat and barley where take-all (causal fungus: *Gaeumannomyces graminis* var. *tritici*, Ggt) was building up were isolated and identified. Several antagonistic fungi including *Alternaria* spp. and *Idriella* spp. were increased by the seed treatment fungicide fluquinconazole. *In vitro*, Ggt and closely related *Gaeumannomyces-Phialophora* species were most sensitive to fluquinconazole. *Epicoccum purpurascens* suppressed take-all most effectively on dual-inoculated wheat plants grown from treated seed in pots.

INTRODUCTION

Take-all of cereals caused by the root-infecting fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) is subject to suppression by other components of the root microflora. These include fungi such as *Phialophora graminicola* (Pg) that can build up in previous grass leys (Deacon, 1973), *Gaeumannomyces graminis* var. *graminis* (Ggg) that has been proposed as a biological control agent (Wong *et al.*, 1996), and microorganisms such as *Trichoderma* spp. that are thought to be involved in take-all decline that often occurs in continuous cereal cropping (Simon & Sivasithamparam, 1989). The imminent introduction of seed treatment fungicides such as fluquinconazole (Mielke, 1997) to control take-all raises the possibility that they will affect fungal antagonists and impair the natural suppression of take-all.

MATERIALS AND METHODS

Root and stem-base pieces from wheat and barley grown as second cereals, treated or untreated with fluquinconazole (4.5 ml/kg seed), sampled in January and June, were serially washed 20 times in cold sterile water and incubated on PDA and low nutrient agar (SNA). All sporulating fungi were identified. Common fungi, as well as Ggt, Pg and Ggg, were tested on PDA for sensitivity to fluquinconazole in a dilution series to determine EC50s. They were also tested for inhibitory activity against take-all in dual-inoculation pot experiments on wheat seedlings grown in sand from seed treated or untreated with fluquinconazole. The percentage of roots infected with Ggt and the lengths of healthy root above Ggt lesions were assessed.

RESULTS AND DISCUSSION

In the field sample taken in June, 30 fungal genera were identified on wheat roots and 28 on barley. *Idriella* spp. on wheat were not affected by seed treatment in January, but in June they increased (from 89.2% to 100% of roots); on barley in January they increased (from 52.5% to 75.8% of roots), but in June they were not affected (SNA). On stem bases, *Alternaria* spp. were not affected in January, but in June they increased on wheat (from 15.4% to 37.1% of

roots) and on barley (from 17.5% to 34.6% of roots) (PDA). Seed treatment decreased the percentage of plants with take-all from 33.8% to 23.7% and increased the grain yield of wheat from 7.7 to 8.8 t/ha by controlling leaf diseases. Take-all was decreased more on dual-inoculated plants grown from fluquinconazole-treated seed than when either an antagonistic fungus (unrelated to Ggt) or the fungicide was present alone (Table 1).

Table 1. Effects of some cereal root fungi on take-all of wheat in dual-inoculated pot experiments and their sensitivity to fluquinoconazole.

Fungal species	% roots blackened		Length of healthy root (mm)		EC_{50} (μg ml^{-1}) ±SE fluquinconazole
	un-treated	fluquin-conazole	un-treated	fluquin-conazole	
Alternaria infectoria	68.7	62.5	5.3	20.2	1.317 ± 0.3803
Epicoccum purpurascens	65.2	52.9	8.1	23.1	0.514 ± 0.0915
Fusarium culmorum	72.2	69.5	5.2	18.5	>20
Idriella bolleyi	74.8	64.8	5.3	19.0	1.404 ± 0.2510
G. graminis var. *tritici* (only)	74.6	72.1	2.5	17.1	0.016 ± 0.0021
SED (232 d.f.)	2.99		1.95		
G. graminis var. *graminis*	71.8	66.0	11.3	21.6	0.059 ± 0.0059
Phialophora graminicola	70.5	61.7	14.2	21.9	0.006 ± 0.0015
G. graminis var. *tritici* (only)	70.4	60.5	6.4	21.3	0.016 ± 0.0021
SED (232 d.f.)	3.04		1.92		

Fluquinconazole has selective activity, being less fungitoxic to some non-pathogenic root fungi than to the *Gaeumannomyces-Phialophora* complex of fungi (Table 1). Except where take-all suppression is caused by fungi in this complex, fluquinconazole is unlikely to impair natural suppression caused by fungi. The model system (dual inoculations) suggests that the efficacy of fluquinconazole may sometimes be enhanced by its specificity for Ggt. Effects in the field are being investigated further in wheat and barley grown as third and fourth cereals.

ACKNOWLEDEGMENTS

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REFERENCES

Deacon J W (1973). Control of the take-all fungus by grass leys in intensive cereal cropping. *Plant Pathology* **22**, 88-94.

Mielke H (1997). Untersuchungen zur Bekämpfung der Schwarzbeinigkeit im Getreide durch Saatgutbehandlung. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **49**, 300-304.

Simon A; Sivasithamparam K (1989). Pathogen suppression: a case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Soil Biology and Biochemistry* **21**, 331-337.

Wong P T W; Mead J A; Holley M P (1996). Enhanced field control of wheat take-all using cold tolerant isolates of *Gaeumannomyces graminis* var. *graminis* and *Phialophora* sp. (lobed hyphopodia). *Plant Pathology* **45**, 285-293.

Studies investigating the effects of propamocarb hydrochloride on the production of oospores of *Phytophthora infestans in planta*

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ABSTRACT

The formation of oospores of *Phytophthora infestans* is inhibited by propamocarb hydrochloride in both leaf disks and whole plants. A rate of 100 µg/µl inhibited oosporogenesis in leaf disks, while no oospores were produced when whole plants were sprayed with 50% field rate.

INTRODUCTION

Oospores of *Phytophthora infestans* were first found in nature in Mexico, near the centre of origin of *Solanum* species (Niederhauser, 1956). The A2 mating type was absent from Europe until the late 1970s, but its introduction has enabled the addition of the sexual stage to the life-cycle of *P. infestans* in Europe. It has been shown that this has produced a general increase in genetic diversity (Drenth *et al.*, 1994), so resulting in increased virulence diversity, the appearance of new virulence factors and a possible enhanced ability to adapt to environmental changes.

Propamocarb hydrochloride is a systemic carbamate fungicide sold in mixture with mancozeb as Tattoo; or with chlorothalonil as Tattoo C for the control of *P. infestans*. It has been reported by Reiter (1994) that propamocarb hydrochloride has a strong inhibitory affect on the sporulation of *P. infestans* while also reducing the sexual reproduction of the fungus. The aim of this work was to examine the effects of propamocarb on oospore production in the plant.

METHODS AND MATERIALS

The production of oospores in the presence of propamocarb hydrochloride was tested in leaf disks and in whole plants.

Leaf disk study

Propamocarb solutions of 0, 1, 2, 5, 10, 20, 50 and 100µg/µl were prepared and 2ml of solution was added to each well of a 25-compartment square repli-dish. 1.5cm potato leaf disks were floated on the propamocarb solution and inoculated with 20µl of a mixed sporangial suspension (2×10^4 spores/ml), with A1 and A2 isolates in equal proportion. Six separate crosses were made with five replicates of each cross. The replidishes were incubated for 4 weeks to allow oospore formation. The number of oospores in each leaf disk was assessed by either (1) a complete count, when oospores appeared to be at a low frequency (0-500 per disk) or (2) by estimating the number of oospores present.

Whole plant study

Propamocarb hydrochloride solution was sprayed onto five week old potato plants (cv. Home Guard) at 200 litres/ha at concentrations of 0, 600, 900 and 1200 µg/µl. After 24h the plants were sprayed with a mixed suspension of A1 and A2 isolates (2 x 10^4 spores/ml, equal proportions), and incubated for 10 days under plastic hoods at near 100% r.h.. After two weeks the number of oospores in a leaf was estimated by first measuring the surface area of the leaf using a digital image analyser, and then liquifying the leaf in a glass grinder in a known volume of water. The number of oospores in 50µl was counted and the total number in the leaf calculated.

RESULTS

Leaf disk study

Table 1. The effect of propamocarb on oospore production in potato leaf disks.

	Mean number of oospores produced per leaf disk in presence of propamocarb							
Concentration of Propamocarb (µg /µl)	0	1	2	5	10	20	50	100
cross 1	3728	3896	2862	1776	1208	444	16	0
cross 2	2776	2305	2790	2096	258	143	8	0
cross 3	4123	3109	3429	1289	1038	288	25	0
cross 4	3710	2785	1320	72	51	0	0	0
cross 5	2632	2576	1048	109	22	0	0	0
cross 6	4022	3232	2107	470	0	0	0	0

It can be seen that propamocarb-HCl has a strong inhibitory effect on oosporogenesis in leaf disks. In crosses 4, 5 and 6 oospore production is completely inhibited at 20 µg/µl, while being significantly reduced in crosses 1, 2 and 3. There was no oospore production at all at 100 µg/µl.

Whole plant study

Oospores were formed in leaf material, petioles and stems in the control plants, but no oospores were found in the 600, 900 or 1200 µg/µl treatments.

REFERENCES

Drenth A; Tas, ICQ; Govers F (1994). DNA fingerprinting uncovers a new sexually reproducing population on *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology* **100**, 97-107.

Niederhauser J S (1956) The blight, the blighter, and the blighted. *Transactions of the New York Academy of Science* **19**, 55-63.

Reiter B (1994) On the mode of action of propamocarbHCl against *Phytophthora infestans* on potatoes and tomatoes. Summary of PhD thesis. University of Hanover.

Improving quality and quantity of the biopesticide *Ulocladium atrum* to enhance biological control of *Botrytis cinerea*

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ABSTRACT

Water-stressed spores of *Ulocladium atrum* contained higher concentrations of polyhydric alcohols (polyols) than those that were unstressed. The modified spores were significantly larger, produced more germ-tubes and had a significantly higher germ-tube extension rate, than unmodified spores at low water availability. In solid-state fermentation, sporulation was increased from $5x10^6$ to $3x10^7$ spores/g dry media, by manipulation of temperature and water potential (MPa). Similarly, in liquid fermentation, production of inocula increased from 1.5×10^6 to 2.7×10^6 when water potential was modified.

INTRODUCTION

Botrytis cinerea (Grey mold) causes economic losses in many commercially important crops and therefore makes an ideal target pathogen for biological control research. The biopesticide *U. atrum* has shown potential in controlling the pathogen by suppressing its sporulation in necrotic plant tissue (Kohl *et al.*, 1995), and consequently reducing severity of disease epidemics. In order for the biopesticide to be used commercially, it is important that two major limitations to successful biological control are overcome: (1) low tolerance of environmental stresses by inocula, and (2) economic production of ecologically competent inocula. The current work aimed to increase quality as well as quantity of *U. atrum* inocula.

METHODS AND MATERIALS

Cultures of *U. atrum* were grown over a 30d period on oatmeal agar modified with various ionic and non-ionic solutes. Spores from the cultures were harvested, plated and grown on water-stressed media before being assessed for spore and germination characteristics with light microscopy and image analysis. Water-stressed spores were also extracted and subjected to high performance liquid chromatography (HPLC) analysis to measure concentrations of endogenous reserves (Hallsworth & Magan, 1994). Production of inocula in water-stressed solid, and liquid fermentation, was assessed by haemocytometer and colony forming unit (CFU) counts.

RESULTS AND DISCUSSION

Quality of modified spores was increased in that they were significantly larger (17 and 24 µm for unmodified and water-stressed spores, respectively) and produced germ-tubes that had a significantly higher extension rate than unmodified spores (100 and 200 µm for unmodified and water-stressed spores, respectively) at low water availability. They also produced significantly more germ-tubes (2.6 and 3.7 for unmodified and water-stressed spores respectively). The modified spores contained higher concentrations of polyols (Figure.1) but lower concentrations of the sugars glucose and trehalose.

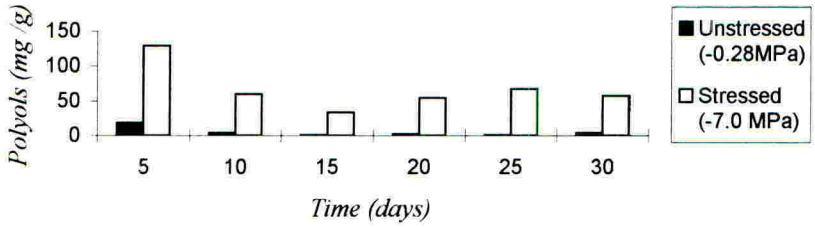

Figure 1. Temporal accumulation of polyols in characterised *U.atrum* spores at 20°C.

The effects of manipulating water stress levels and temperature on production of *U. atrum* inocula are compared in Table. 1 The data clearly show that production is enhanced when water potential of the media is modified.

Table 1. Effect of temperature and water potential on production of *U.atrum* inocula.

Media water potential	Spores/g dry media after 3 weeks		
Solid substrate	20°C	25°C	30°C
-0.28	1.1×10^7	7.7×10^6	7.4×10^6
-1.4	7.0×10^6	2.3×10^7	2.4×10^7
-2.1	1.5×10^7	1.6×10^7	5.6×10^6
-3.0	6.8×10^6	9.1×10^6	3.0×10^7
Liquid media	Spores and mycelial fragments/ml after 1 week		
-0.28		1.5×10^6	
-2.1		2.7×10^6	

Research is continuing in order to define more fully the optimum conditions for inoculum production, and to evaluate the quality of the modified biological control propagules.

REFERENCES

Hallsworth J E; Magan N (1994). Improved biological control by changing polyols /trehalose in conidia of entomopathogens. In: *Proceedings of the Brighton Crop Protection Conference. Pests and Diseases*. 1091-1096.

Kohl J; Molhoek W M L; van der Plas C H; Fokkema N J (1995) Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field conditions. *Phytopathology* **85**, 393-401.

A biorational approach to selecting mycoinsecticides for aphid management

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ABSTRACT

In laboratory bioassays, Hyphomycete fungi showed differential virulence against *Aphis fabae*, *Coccinella septempunctata* and *Praon volucre*. *In vitro* studies indicated that some species of fungi grew and germinated better than others at low temperatures (10°C). This information was used to select isolates for field testing.

INTRODUCTION

Fungi have been identified as potential biological control agents against aphids in arable crops (Vandenberg, 1996). Although virulence against the target insect is important, we are also considering the impact of isolates on non-target beneficials and isolate interactions with the abiotic environment. By taking this approach we will select isolates that will be compatible with both the biotic and abiotic environment in which they will be used.

MATERIALS AND METHODS

To investigate the impact of temperature on *in vitro* growth of fungi, single plugs of each isolate were placed onto media and incubated at four temperatures; 10, 15, 20 and 25°C. The rate of radial growth of each fungal colony was measured. Data were analysed using an ante-dependence test (Kenward, 1987).

Isolates were screened in a single-dose bioassay against *A. fabae*. Aphids were sprayed with suspensions of fungi ($1x10^8$ conidia/ml in 0.03% Tween 80) or Tween (0.03%) as a control. Spray applications were made using an electrostatic rotary atomiser on a track sprayer at 0.4m/s applying 24 ml/min which equates to 10.4 litre/ha. Mortalities were monitored daily and data were analysed using the Kaplan-Meier method for survival data.

Adult *C. septempunctata* and *P. volucre* were treated with fungal suspensions ($1x10^8$ conidia/ml in 0.03% Tween 80) or with Tween 80 (0.03%) and daily mortalities recorded. Fungal isolates were simultaneously screened against *A. fabae* as a positive control.

RESULTS

There was a significant difference in the radial growth of all isolates at each temperature after

the first 2-4 days of growth ($P<0.001$). Trends suggested that *Paecilomyces fumosoroseus* isolates were the most tolerant to the range of temperatures. Preliminary results from *in vitro* germination experiments showed similar trends.

The total number of aphids treated which were killed by fungus in bioassays ranged from 53% for isolate T229 (*Paecilomyces farinosus*) to 100% for isolate 1.72 (*Verticillium lecanii*). The isolates which resulted in the lowest median survival time (MST) of aphids were selected from each bioassay. These isolates were; 1.72 (*V. lecanii*; MST=3.7 days), 2879 (*Beauveria bassiana*; MST=4.4 days), Z11 (*P. fumosoroseus*; MST=3.7 days) and 'Mycotech' *B. bassiana* strain GHA (MST=5.0 days). Subsequent field testing was conducted with isolates 1.72 *V. lecanii* and 'Mycotech' *B. bassiana* strain GHA.

In bioassays, there were large differences between isolates in their virulence towards *C. septempunctata* (Table 1). The percentage of adult *P. volucre* emerging from treated mummies was 76% for controls and 85% for treated mummies. However, 73% of those insects that emerged from treated mummies succumbed to fungal infection.

Table 1. Virulence of seven isolates in bioassays against *C. septempunctata* and corresponding virulence against *A. fabae*.

Fungal Isolate	Infection (%)	
	C. septempunctata (n=30)	*A. fabae* (n=72)
GHA (*B. bassiana*)	21	75
"	20	94
"	90	92
T195 (*B. bassiana*)	71	79
T130 (*Metarhizium anisopliae*)	100	96
2859 (*V. lecanii*)	7	94
Z4 (*P. fumosoroseus*)	3	78
T229 (*P. farinosus*)	3	26
T80 (*V. lecanii*)	0	92

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REFERENCES

Kenward M G (1987). A method for comparing profiles of repeated measures. *Applied Statistics* **36**, 296-308.

Vandenberg J D (1996). Standardised bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against the Russian wheat aphid (Homoptera: Aphididae). *Journal of Economic Entomology* **89**, 1418-1423.

A study of olfactory and visual cues attracting the sweet potato butterfly, *Acraea acerata*, to its host plant

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ABSTRACT

The results of a bioassay in a wind tunnel using glass-screened, muslin-screened and non-screened sweet potato plants suggest that sweet potato volatiles play an important role in attracting *Acraea acerata* to its host plant. Both the distance moved by mated female butterflies towards muslin-screened plants (olfactory cues) and the percentage of butterflies which landed on the screen support this conclusion. Visual cues seemed to have a negative effect.

INTRODUCTION

The sweet potato butterfly (*Acraea acerata*) is a serious pest of sweet potato in East Africa. Its caterpillars defoliate sweet potato leaves reducing tuber yield by up to 70% (Tardif-Douglin & Rwalinda, 1993). Small subsistence farmers who rely on sweet potato as a staple crop cannot easily afford to buy pesticides with which to control the butterfly. Such farmers could potentially use intercropping, the most common cultural practice of the tropics (Altieri, 1994), to reduce damage from the butterfly. Intercropping tends to disrupt and/or mask plant olfactory and/or visual stimuli which attract the host plant seeking insect pests (Altieri, 1994).

With the aim of developing a pest management strategy for sweet potato butterfly using appropriate intercropped plants, we have initially examined the role of plant visual structures and olfactory volatiles in attracting *A. acerata* to its host plant.

MATERIALS AND METHODS

Sweet potato plants were grown in the glasshouse using vine cuttings planted in pots. All the plants used for the bioassay were from the same clone. Butterflies were kept between 23-27 °C with a photoperiod of L12:D12 and 70% relative humidity. Eggs of *A. acerata* obtained from Uganda had been collected from a sweet potato field. Caterpillars were fed on sweet potato plants and butterflies on a 10% sugar solution.

The experiment was carried out in the wind tunnel described by Hern (1997). Its experimental area measured 2.0 m wide, 1.75 m long and 1.0 m high. The lighting provided full spectrum light close to daylight. The average wind speed was 29 cm/s and the temperature interval was 26-30 °C. Treatments were introduced to the wind tunnel to allow the wind speed and the temperature to stabilise before releasing butterflies. There were 20 butterflies per treatment.

There were four treatments: pots with peat only (control), glass-screened (visual cues), muslin-screened (olfactory cues) and non-screened sweet potato plants (visual & olfactory cues). Six week old plants were used. For each treatment, three pots were placed at about 25 cm from the upwind wall of the tunnel. Individual two day old naive and mated female butterflies were released onto a thread through a small window at the height of 35 cm in the downwind wall of the tunnel. The main behavioural events recorded were resting, walking, flying and landing on the treatment for a total observation time of 30 minutes per individual butterfly. The positions of butterflies in wind tunnel were also recorded.

RESULTS AND DISCUSSION

The analysis of variance of the average distances (cm) moved by sweet potato butterflies towards the treatments suggests a very strong effect of sweet potato volatiles (33.87 ± 6.92, $P < 0.001$; 50.52 ± 8.93, $P < 0.001$; 51.10 ± 9.17, $P < 0.001$ respectively for the periods 1-10, 11-20 and 21-30 minutes) in attracting the butterflies towards their host plant. The effect of visual cues (-19.27 ± 6.92, $P = 0.007$) and subsequently the effect of the interaction of both visual and olfactory cues (-39.42 ± 13.87, $P = 0.006$) on movement towards the host plant seemed to be negative for the first 10 minutes of the observation period. These results, supported by the very high percentage of butterflies which landed on the muslin screen (Figure 1), suggest that female sweet potato butterflies are stimulated by host plant volatiles to orient and move upwind towards their host plants.

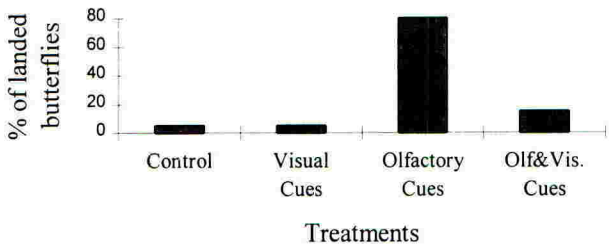

Figure 1. Landing of butterflies in wind tunnel

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REFERENCES

Altieri M A (1994). *Biodiversity and pest management in agroecosystems*. 180 p. The Haworth Press, Inc., Binghamton, NY.

Hern A (1997). *Aspects of pre-oviposition behavior of Pieris rapae*. PhD thesis, University of Edinburgh, UK.

Tardif-Douglin D; Rwalinda P (1993). *Situation de la patate douce et du manioc au Rwanda: Réflexion sur leur production, productivité et leur perspective d' avenir dans l' agriculture rwandaise*. Publication DSA N° 26, Minagri, Kigali, Rwanda.

Tebufenozide and methoxyfenozide against the beet armyworm, *Spodoptera exigua*

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ABSTRACT

Toxicity of the ecdysone agonists, tebufenozide and methoxyfenozide (RH-2485), was measured by feeding treated diet to laboratory strain larvae of the beet armyworm, *Spodoptera exigua* (Hübner). Ingestion of enzyme synergists along with ecdysone agonist indicated the importance of oxidative metabolism. Mono-oxygenase activities were measured in the whole body and midgut of last-instars and in adults.

INTRODUCTION

The beet armyworm, *Spodoptera exigua* (Hübner) is a polyphagous noctuid pest of worldwide importance in agriculture, horticulture and ornamentals. In various places, failure of control has been reported due to resistance to or reduced efficacy of several insecticides as organophosphates (OPs), carbamates, pyrethroids, benzoylphenyl ureas and *Bacillus thuringiensis* formulations.

Tebufenozide is the first commercially available non-steroidal ecdysone agonist. Methoxyfenozide (RH-2485) is a novel structural analogue which is expected to be more lepidopteran specific and more toxic, and which might be introduced on the market in 1999. These compounds manifest their toxic effects, especially in Lepidoptera, via interaction with the ecdysteroid receptor (Dhadialla *et al.*, 1998).

METHODS AND MATERIALS

All stages of *S. exigua* were maintained at standard conditions (Smagghe & Degheele, 1994).

Tebufenozide and methoxyfenozide (courtesy of Rohm & Haas, USA) were supplied to newly moulted last-instars via artificial diet; mortality was scored at 7 DAT and subjected to probit analysis (Smagghe & Degheele, 1994). Piperonyl butoxide (PB) (Fluka, Belgium) and *S,S,S*-tributyl phosphorotrithioate (DEF) (courtesy of Bayer, USA) were tested as enzyme synergists. Percentages were corrected for mortality in controls (Smagghe *et al.*, 1998).

Enzyme activity of mono-oxygenases in the whole body and midgut of last-instars and in whole adults was spectrophotometrically measured with *p*-nitroanisol (Van Laecke *et al.*, 1995).

RESULTS AND DISCUSSION

Based on LC_{50}s, methoxyfenozide (0.38 ppm) was nearly 2 times more toxic than tebufenozide (0.60 ppm). The current toxicity values concur with the findings in various insect pests (Smagghe & Degheele, 1994; Dhadialla *et al.*, 1998), suggesting these compounds to be useful for controlling caterpillars.

PB synergized the toxicity of ecdysone agonist, increasing the mortality from 54% with 0.4 ppm methoxyfenozide alone to 91% (synergism ratio SR=1.7) with addition of 100 ppm PB (Fig. 1). Recently, we reported that application of tebufenozide+PB resulted in an LC_{50} of 0.18 ppm as compared

to 0.58 ppm with tebufenozide alone (SR=3.4) (Smagghe *et al.*, 1998). Addition of DEF to methoxyfenozide and tebufenozide resulted in respective SR values of 1.2 and 1.5.

The majority of oxygenases in larvae was present in the larval midgut, and that in the total body of last-instar larvae was about 2-fold higher than in adults (Table 1). This proves the gut to be a primary source of degradative enzymes against insecticides.

Table 1. Mono-oxygenase activity (nmol/mg protein.min) in *Spodoptera exigua*.

Tissue	Enzyme activity	Ratio
Larval body	9.6	1
Larval midgut	553.1	57.6
Adult body	5.0	1.9

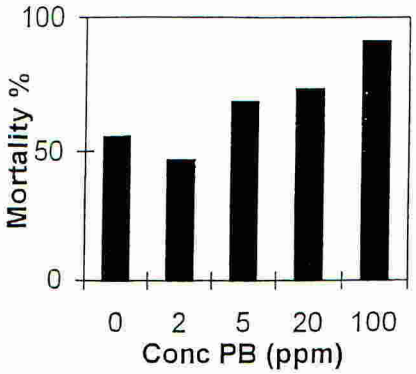

Figure. 1. Synergism of 0.4 ppm methoxyfenozide with different concentrations of PB.

It is a well known phenomenon that an increased metabolic activity in insects is an important mechanism of insecticide resistance. Here, synergism of PB suggested that the major first phase route of detoxification is through oxidation. This indicates that PB can be useful to increase the toxicity of ecdysone agonists and to screen for enhanced oxidase levels in various resistant specimens. The results obtained so far indicated that these enzymes are predominantly present in the larval midgut, which makes this insect tissue an interesting target tool for evaluation. Hence, P_{450} mono-oxygenases are known to be important in resistance development towards other groups of insecticides such as pyrethroids, OPs and carbamates (McKenzie, 1996). So, cross resistance with other insecticides should be carefully analyzed. These lines of research are in progress in our laboratory and aim to provide guidelines for resistance management of this group of insect growth regulators in the future.

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REFERENCES

Dhadialla T S; Carlson G R; Le D P (1998). New insecticides with ecdysteroidal and juvenile hormone activity. *Annual Review of Entomology* **43,** 545-569.

McKenzie JA (1996). Ecological and evolutionary aspects of insecticide resistance. Academic Press, Environmental Intelligence Unit, R.G. Landes Co., Austin, TX, USA, 185 p.

Smagghe G; Degheele D (1994). Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. *Pesticide Science* **42,** 85-92.

Smagghe G; Dhadialla T S; Derycke S; Tirry L; Degheele D (1998). Action of the ecdysteroid agonist tebufenozide in susceptible and artificially selected beet armyworm. *Pesticide Science* in press.

Van Laecke K; Smagghe G; Degheele D (1995). Detoxifying enzymes in greenhouse and laboratory strain of beet armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **88,** 777-781.

Biodegradation of the nematicide ethoprophos in soils from the UK and Greece

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ABSTRACT

Soil samples from the UK with no history of ethoprophos application were treated three times with ethoprophos at three dose levels: 1.5, 5 and 15 mg a.i./kg dry soil. There was a gradual decrease in degradation rate of each successive application. Soil samples were also collected from adjacent field sites in Greece that had either been treated with ethoprophos or had no history of ethoprophos use. The times for 50% loss of a fresh ethoprophos addition were approximately 4 and 40 days for the previously-treated and untreated soils, respectively. An addition (5%) of this enhanced soil to the UK soil containing aged residues of ethoprophos resulted in degradation of both a fresh application plus the aged residues within 28 days. When the enhanced soil was sterilized prior to mixing into the UK soil, over 40% of ethoprophos residues were present after 42 days.

INTRODUCTION

There is evidence that many pesticides are subject to the phenomenon of enhanced biodegradation in soil following their repeated application at the same site. In its extreme form, this can lead to reduced efficacy, as with carbofuran and iprodione. Ethoprophos is used in Greece and the UK for the control of potato cyst nematode *(Globodera rostochiensis)*. Previous studies have suggested that its efficacy can be reduced in previously treated fields due to accelerated biodegradation. The aim of this study was to examine the development of enhanced degradation of ethoprophos in laboratory and field treated soils and to investigate the biological involvement in its rapid degradation.

MATERIALS AND METHODS

Soil samples from HRI, Wellesbourne with no history of ethoprophos use were treated three times at intervals of 8 to 10 weeks with 1.5, 5 or 15 mg a.i./kg ethoprophos. Samples (1 kg) were incubated at 15°C and residues of parent compound were measured at intervals by methanol extraction of sub-samples (20g) followed by GLC with an NPD detector. Soils with known pretreatment histories of ethoprophos were collected from Greece. Ethoprophos was incubated in these soils and its degradation rate measured. Samples of the UK soil which had received three applications of ethoprophos were treated with (a) 5% of the previously treated soil from Greece, (b) 5% of the soil from Greece following its sterilization, and (c) no addition. All samples received 5 mg a.i./kg ethoprophos and its degradation rate was determined.

RESULTS AND DISCUSSION

There was no increase in degradation rate of ethoprophos in the UK soil following repeated applications at any of the dose levels tested (Figure 1). Instead, a gradual accumulation of residues occurred which, at the highest concentration examined, was coupled with a significant decrease in the size of the microbial biomass. The lack of acceleration after three repeated applications in the laboratory may be attributed to either inhibitory effects of high ethoprophos residues on the soil microflora or to the low pH of the soil (5.2) which would not favour the development of enhanced biodegradation.

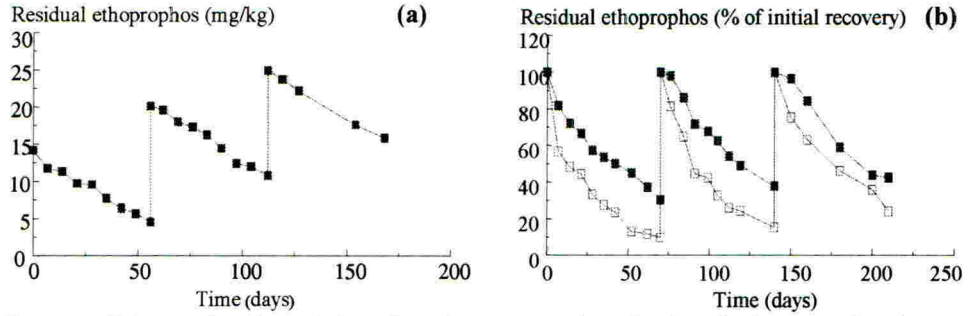

Figure 1. Ethoprophos degradation after three repeated applications in the UK soil at three dose levels; (a) 15 (b) 5 (■) and 1.5 (□) mg/kg.

Rapid loss of ethoprophos occurred in the Greek soil previously treated with ethoprophos in the field (Figure 2a). The time for 50% loss of ethoprophos was 10 times smaller than that in the previously-untreated control. Incorporation of 5% enhanced soil from Greece into UK soil which had received three pre-treatments with 5 mg/kg of ethoprophos with no sign of acceleration resulted in degradation of both fresh and aged residues within 28 days (Figure 2b). The degradation rate of ethoprophos was significantly slower in the UK soil treated for the fourth time or mixed with 5% sterilized soil from Greece. These findings indicate the presence of a microbial population in the Greek soil, able to rapidly degrade ethoprophos.

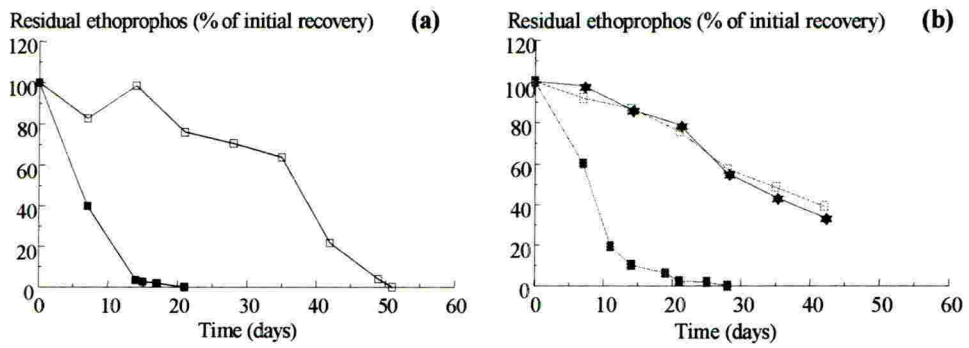

Figure 2. The degradation of ethoprophos (a) in previously treated (■) and untreated (□) soils from Greece, (b) in UK soil mixed with, 5% of previously treated soil from Greece (■), with 5% sterilized previously treated Greek soil (✱), untreated-not mixed (□).

Ground spray coverage study under a field sprayer boom

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ABSTRACT

Laboratory tests were made with a conveyor to study ground spray distribution under a small boom of a field sprayer. The influence of boom height, boom speed, nozzle angle and nozzle type on spray coverage, spray evenness and spray dose was studied. Measured distributions were compared with theoretical ones. All these parameters influenced spray coverage as well as evaporation or spray drift.

INTRODUCTION

There is much public concern regarding agricultural spraying and the use of pesticides. Because of this it is necessary to ensure that agricultural equipment is used correctly in order to reduce the negative impact of spraying on the environment. Several parameters need to be considered for pesticide management (implementation of the International Code of Conduct on the distribution and use of pesticides) and certification of application equipment for pesticides. It is thus essential to study the parameters influencing ground coverage by sprays in order to ensure more accurate spraying and to reduce chemical utilisation (Enfält *et al.*, 1997b).

METHODS AND MATERIALS

Dynamic spray distributions under a boom were obtained with a conveyor moving a small boom containing 8 nozzles at 50-cm spacing. Many possible methods are described in the literature (Salyani & Whitney, 1988) but the image analysis method developed by Enfält *et al.* (1997a) was chosen. Nozzle distributions were measured on a patternator and were processed to give a static 2D spray distribution, knowing the riding velocity (this gave the theoretical distribution without dynamic effect). Distributions are represented with grey levels corresponding to the amount of product collected on 5 × 5 cm^2 areas. These images are threshold to define correctly sprayed, over-sprayed and under sprayed areas: correct spraying areas are defined for cells where the amount of collected product does not exceed 15% from the mean value. Several mathematical tools based on statistics, image analysis and signal processing were used to compare static and dynamic spray distributions.

Measurements of droplet size and velocity made with a Phase Doppler Analyser were added to complete the dynamic effect study.

RESULTS

Staticic uneveness was similar to dynamic spray unevenness (Fig. 1). Diffeences in spray coverage appeared, depending on the nozzle type and, for each nozzle, boom height had an important influence (Figure 2). Unevenness increased for increasing boom heights and decreasing velocities. The amount of collected product decreased with increasing heights and DPA measurements were coupled with a state of the art concerning spraying phenomena as evaporation (Sundaram, 1995), drift, droplet dispersion and droplet speed in several research fields as agricultural spraying, irrigation or forestry applications, to explain it.

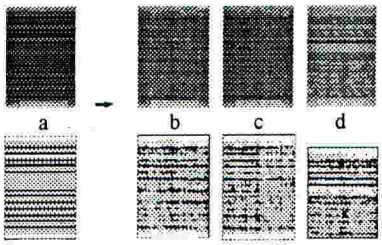

Figure 1. Comparison of static and dynamic 2D distributions.

Boom speed : 6 km/h, boom height : 30 cm, nozzle : Teejet XR 8002VS
Upper figures : Grey level representations. (a) static, (b)(c)(d) dynamic (3 repetitions)
Lower figures : Threshold representations. (e) static, (f)(g)(h) dynamic (3 rep.). Grey : correct spraying, black : over-sprayed, white : under-sprayed

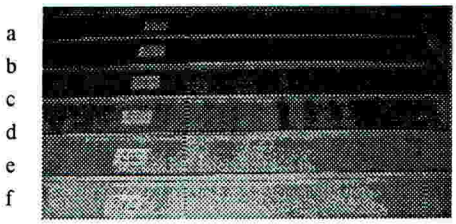

Figure 2. Influence of boom height and nozzle type on the distribution.

Nozzle : Teejet XR 11004VS. Boom height : 30 cm (a), 50 cm (b), 70 cm (c)
Nozzle : Teejet XR 8002VS. Boom height : 30 cm (d), 50 cm (e), 70 cm(f)

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REFERENCES

Enfält P; Engqvist A; Alness K (1997a). Assessment for the dynamic spray distribution on a flat surface using image analysis. *Aspects of Applied Biology* **48**, 17-23.

Enfält P; Engqvist A; Alness K (1997b). The influence of spray distribution and drop size on the dose response of herbicides. *Proceedings of the Brighton Crop Protection Conference - Weeds*, 381-389.

Salyani M; Whitney J D (1988). Evaluation of methodologies for field studies of spray deposition. *Transactions of the ASAE* **31(2)**, 390-395.

Sundaram A (1995). Physical properties and evaporation characteristics of non aqueous insecticide formulations, spray diluents and adjuvant/co-solvent mixtures. *Journal of Environmental Science and Health* **B30(1)**, 113-138.