SESSION 4C

DEVELOPMENTS IN THE MANAGEMENT OF PESTS AND DISEASES IN TROPICAL AGRICULTURE

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THE NEED TO CONTROL PESTICIDE QUALITY IN DEVELOPING COUNTRIES

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ABSTRACT

Pesticide products were analysed from 21 developing countries in Latin America, Africa, and Asia over the period from 1989 to 1994. The results presented and other extensive studies in individual developing countries indicate that over one-third of pesticides available on the market in developing countries do not comply with international standards.
Increased efforts must be made by manufacturers and traders but also by purchasers and users to improve the situation. In addition, national control authorities in developing countries must be enabled to control effectively the quality of products which are registered, imported and marketed.

INTRODUCTION

In developing countries, pesticides are used not only in agricultural production, but also on a large scale in post-harvest and stored-food protection, to improve human, animal and plant health, in households, and to control migratory pests and vectors of disease. Whatever the purpose, the marketed pesticides must be of a quality which complies with the respective national regulations and internationally accepted standards, so that the products can be used safely and effectively.

In November 1985, the FAO Conference adopted an International Code of Conduct on the Distribution and Use of Pesticides, which *inter alia* sets forth quality standards, and describes generally accepted trade practices (FAO, 1986).

A first evaluation of the implementation of the Code's provisions in 1986 revealed that the situation in developing countries still fell far short of expectations. With respect to the quality of pesticides, 46% of the 101 developing countries surveyed indicated that the pesticides marketed in their country did not comply with international specifications. Amongst the countries of Latin America the figure was 34%, in Asia and the Pacific region 43%, and in Africa 62% (FAO, 1993).

PRODUCT QUALITY CONTROL

The Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, together with its partner institutions in developing countries, has been conducting tests on the quality of pesticides for many years, within the scope of development cooperation. Depending on the needs of the local partner agency, tests are carried out on new products, market samples, or outdated products.

Product quality comprises the quality of the active ingredient, the formulation, the packaging and the labelling. The tests described here involve primarily analysis of the active ingredient, this being the most important component of the products.

The results of analyses of a total of 348 product samples were selected from a wealth of data. This included only samples of products intended for direct use in the country in question, which had not yet reached the expiry date specified by the manufacturer. The samples were taken in 21 developing countries in Latin America, Africa and Asia over the period from 1989 to 1994, and were tested at the GTZ-contracted laboratory for pesticide quality control in Schopfheim, Germany. The results of the active ingredient analysis were compared to the active ingredient concentrations as declared on the product label. The tolerance limits set out in the FAO specifications were used as a basis for evaluation (FAO, 1995).

Upon comparison of the declared with the measured active ingredient concentrations, an average of 34% of the tested products were found to be outside the FAO tolerance limits.

The results presented here from tests conducted on a random-sample basis, have been confirmed by extensive studies in individual developing countries (Table 1). In Costa Rica, the figure for products failing to comply with the FAO specifications was 29%, in El Salvador and Panama around 28%, in Madagascar 56%, and in Malaysia 18-37% (Balasubramaniam, 1996; de Alvarado & Bodzian, 1994; von Dueszeln *et al.*, 1995).

Table 1. Tests on product quality in specific developing countries.

Country	Year	Number of analysed samples	Samples not in compliance with FAO (%)
Costa Rica (von Dueszeln *et al.*, 1995)	1992	408	29
El Salvador (de Alvarado & Bodzian, 1994)	1994	71	28
Panama (von Dueszeln *et al.*, 1995)	1993	254	27.6
Madagascar (von Dueszeln *et al.*, 1995)	1992-94	655	56
Malaysia (Balasubramaniam, 1996)	1987-91	396	18-37

CAUSES OF POOR QUALITY

The present paper will consider only pesticides declared to be and available as market products in developing countries. In our experience, possible causes of the inadequate quality of pesticides can lie both in poor production and formulation, and in poor labelling and packaging (the additional problem of out-dated products is not addressed here).

Inadequate purification of the active ingredients in the production process can lead to inadmissible additional ingredients remaining in the product. During formulation, an over-concentration or under-concentration of the active ingredient may occur in the product which is outside the permissible tolerance range.

One critical factor is also the quality of the formulation. If the active ingredient is not sufficiently stabilized in the formulation, the result may be premature decomposition of the products, especially at elevated storage temperatures. Another phenomenon is that liquid formulations display an excessive active ingredient concentration, which may be due to the evaporation of solvents through the container walls resulting from poor quality packaging materials. As pesticides are as a rule not stored under cool conditions in developing countries, the issue of heat stability is of special significance in this context.

One major problem in developing countries alongside the poor quality of the active ingredient and the formulation is the insufficient labelling of products. The labelling often fails to provide data on the active ingredient, application, date of manufacture and safe handling of the chemical. A further problem in developing countries is that pesticides are often decanted from large drums into smaller units; it is then often the case that the smaller containers are inadequately marked or not even marked at all. As a result the user, for whom the label is often the only source of information on the product, lacks the necessary directions for safe and effective use of the chemical. Whilst international guidelines such as the "FAO Guidelines on Good Labelling Practice for Pesticides" (FAO, 1985) do exist, in many developing countries compliance with these international standards is not required or monitored consistently, which means that manufacturers and distributors are able to market inadequately labelled products, either through ignorance or as a deliberate strategy.

CONCLUSIONS

The studies presented indicate that over one-third of pesticides available on the market in developing countries do not comply with international standards for quality of active ingredients. If the quality of labelling and packaging is also taken into account, the proportion of poor-quality products is even higher.

In 1994, the global market for pesticides reached a volume of around US$ 26 billion, of which Africa for instance had a market share of only 2 percent (IVA, 1995). Proceeding on the basis of the estimated value of the sub-standard 34%, this would mean that, in 1994, pesticides worth approximately US$ 175 million which did not comply with international quality standards were on the market in Africa alone. Measured in relation to market volume, the corresponding values for Latin America and Asia would be around six to eight times that figure.

The problem of poor quality products reaching the market is not confined to the pesticide market, but also occurs in other sectors in which chemical products are marketed, the quality of which can only be monitored by means of relatively costly laboratory tests. For instance, the WHO estimates that counterfeit pharmaceutical drugs worth several billion US dollars are sold every year in developing countries (WHO, 1992).

In developing country markets, the percentage of products which do not meet international standards is alarmingly high. All those involved must become aware of the scale of the problem, and make increased efforts to address the following points:

- Purchasers and users must be sensitized to the fact that poor-quality products not only have an inadequate effect, but can also entail a burden or even a risk to human health and the environment. Consequently, when the product is purchased the buyer must take into account not only its nature and price, but also more importantly the quality of the product.

- It is the responsibility of manufacturers and distributors to bring products onto the market which are of good quality. Product responsibility must not be neglected, or transferred to others, either through ignorance or deliberately.

- The responsible national control authorities in developing countries must be enabled to control the quality of products which are registered, imported and marketed. To protect buyers and users, violations of quality standards must be detected and punished without reservation.

There are already a number of laws and internationally-binding guidelines which lay down quality standards for products. In developing countries, however, the mechanisms to implement and effectively control compliance with these standards are still lacking in many cases. Only when these mechanisms are established will it be possible to gain a tighter grip on problems with chemicals in developing countries.

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THE USE OF PYMETROZINE FOR THE CONTROL OF HOMOPTEROUS INSECT PESTS IN PADDY RICE

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ABSTRACT

Pymetrozine gave excellent control of planthoppers in paddy rice under field conditions. However, neither topical nor root systemic treatments led to high mortality under laboratory conditions. Contact treatment with pymetrozine caused paralysis of the legs of treated *Nilaparvata lugens* and stopped their sucking behaviour. More than half of the treated individuals died from direct poisoning. The survivors did not contribute to reproduction, primarily because of reduced fecundity caused by delayed ovary maturation. This effect was more obvious in immature than mature females. Pymetrozine suppressed the hopper populations of *Sogatella furcifera* efficiently in the field by both nursery box (systemic) and foliar applications. The duration of its population-suppressive activity against *S. furcifera* in the field was longer than 2 months by nursery box application at the dose of 300 g a.i. /ha and 4 weeks by foliar spray at the dose of 63 g a.i./ha. Pymetrozine also inhibited walking behaviour of the stink bug, *Eysarcoris lewisi*.

INTRODUCTION

The brown plant hopper (BPH: *Nilaparvata lugens*), the white-backed plant hopper (WBPH: *Sogatella furcifera*), the smaller brown plant hopper (SBPH: *Laodelphax striatellus*) and the green rice leafhopper (GRLH: *Nephotettix cincticeps*) are important homopterous insect pests of rice in Japan. BPH and WBPH migrate to Japan from eastern parts of the Asian continent every year and their outbreaks often cause serious yield loss, particularly in west Japan. SBPH and GRLH are indigenous to Japan and are important as vectors of rice stripe virus and rice dwarf virus, respectively. Stink bugs, e.g. *Eysarcoris lewisi*, are also of importance as the dark spots on grains resulting from their feeding reduce the market value of grains.

Pymetrozine is a new insecticide discovered and developed by Ciba. It provides excellent control of aphids, whiteflies and hoppers, with a novel mode of action through inhibition of sucking (Flückiger *et al.*, 1992 a,b;, Kayser *et al.*, 1994). In Japan, Ciba-Geigy Japan, Tomono Agrica and Hokko Chemical Industry are developing the product for use in paddy rice. In these developmental studies, pymetrozine demonstrated novel action and excellent control of important plant hoppers. This paper describes the insecticidal performance of pymetrozine and its mode of action against homopterous insect pests in rice under laboratory and field conditions.

MATERIALS AND METHODS

Laboratory tests

Nilaparvata lugens (BPH), *Sogatella furcifera* (WBPH) and *Nephotettix cincticeps* (GRLH) were reared on rice seedlings at 25±2°C. *Eysarcoris lewisi* was reared on rice grains and peanuts at 25±2°C. Rice plants (*Oryza sativa)*, cv. Nipponbare were used throughout.

Insecticidal and knock-down activity:

Topical application: A stock solution of pymetrozine in dimethyl-sulfoxide was appropriately diluted with acetone. Female adults of BPH, 24 to 48 hour old macropterae, were anaesthetized lightly with carbon dioxide. Pymetrozine was topically applied on the dorsal thorax of each insect in a volume of 0.25µl with a hand micro-applicator. Treated insects were put into glass tubes with rice seedlings.

Systemic application: Rice plants (4th to 5th leaf stage), the roots of which had been washed with distilled water, were placed in an aqueous solution of pymetrozine containing Triton X-100 at 100ppm. Each rice stem was covered with a small wire cage. Ten female adults of BPH, 24 to 48 hour old macropterae, were put into the cage.

Knock-down activity: The low-drift dust formulation of pymetrozine (0.5%, 200g a.i./ha) was applied to potted rice plants grown under submerged conditions and infested with female adults of BPH and GRLH.

Effect on feeding behaviour and reproduction:

Immature, 24 to 48 hour-old, and mature, 7 day-old, female and male macropterae adults of BPH were treated with pymetrozine topical application as described in the insecticidal activity test. Each treated pair was put into a glass tube with a rice seedling. The amount of sugar in secreted honeydew of the survivors was quantified as a parameter of feeding using the anthrone reaction (Dreywood, 1946 ; Abe *et al.*, 1968). The number of eggs laid by each mature female was counted on 1, 2 and 3 days after treatment. To check the fecundity of the treated female adults, 5 specimens were sampled at random and dissected to count mature eggs in their ovaries.

Effect on walking speed of a stink bug:

Female adults of *Eysarcoris lewisi* were treated with pymetrozine by topical application to the dorsal thorax of each insect in a volume of 1 µl, via a hand micro-applicator. Each treated insect was put into a glass tube with three rice grains. Three, 6, 24, 48 and 72 hours after treatment, their walking speed was evaluated as time (seconds) for which they moved 50 mm on a filter paper.

Field trials

Nursery box application: Fifty grams of pymetrozine (3% granule) were applied, 3 hours before transplanting, on the surface of a rice nursery box (58 cm in length x 29 cm in width x 1.5 cm in depth) containing third-leaf stage seedlings (sufficient to cover 50 m^2 of paddy field). The treated seedlings were transplanted into the paddy field with a planting machine. The number of infesting insects was counted by beating the plants and counting dislodged insects.

Foliar application: The low-drift dust formulation of pymetrozine (0.5% dust) was applied with a portable duster. An aqueous solution of the wettable powder (25%WP) was sprayed at the volume of 1000 litres/ha, using a knapsack type power sprayer. The number of infesting insects was counted by the beating method.

RESULTS AND DISCUSSION

Laboratory tests - Insecticidal and knock-down activity

Only moderate BPH mortality was obtained at the high dose of 2.5µg/insect by topical application (Table 1). Insecticidal activity through root uptake was not particularly good even at the high dose of 100 ppm (Table 2). However, interesting effects were observed after the topical treatment: the hind-legs of the treated insects were paralysed, and their reactions apparently delayed. Most of the treated individuals fell to the bottom of the glass tube from rice seedlings. These effects are considered to be a primary contribution to insecticidal efficacy in the field.

Table 1. Insecticidal activity against BPH by topical application of pymetrozine

Dose	Days after application (DAA) and % mortality		
(µg / insect)	1	2	3 DAA
2.5	35	80	85
0.25	5	15	15

Table 2. Insecticidal activity of pymetrozine against BPH by root dipping

Concn.	Days after application (DAA) and % mortality				
(ppm)	1	2	3	4	5 DAA
100	25	40	45	55	55
10	20	20	20	20	30
1	15	15	15	15	30

Pymetrozine dust formulation (0.5%) demonstrated rapid knock-down activity against BPH and GRLH at the dose of 200 g a.i./ha (Table 3). Most treated insects were paralysed and about 50% of them fell down onto the water surface within the first hour. These could no longer climb up the rice stems and soon drowned.

Table 3. Knock-down activity against BPH and GRLH by direct dusting with pymetrozine (0.5% dust, equivalent to 200 g a.i.ha)

Insects	% knock-down and (% mortality)				
	1	4	24	48	DAA
BPH	50 (15)	73 (59)	88 (85)	88	(85)
GRLH	58 (3)	89 (72)	92 (92)	100	(100)

Effect on feeding

The amount of sugar in honey dew excreted by survivors after topical application was significantly less than that of control (Table 4), proving that pymetrozine suppressed feeding of BPH. This effect was more obvious in immature adults.

Table 4. Effect of topical application on feeding by BPH

Insecticides	Dose	Amount of sugar in honey dew micro-grams / pair					
	µg / insect	Immature adults			Mature adults		
		1	2	3	1	2	3 DAA
Pymetrozine	2.5	0.96a	1.22a	3.82a	0.60a	4.20a	4.20a
	0.25	0.70a	5.30b	8.50a	0.58a	3.18a	4.12a
Control	-	4.60b	14.20c	25.80b	2.60b	4.60a	7.20a

Means with differing letters are significantly different (P<0.05)

Effect on reproduction

Mature female BPHs treated with pymetrozine at 2.5 µg/insect laid few eggs for the first 2 days, but the inhibitory effect was completely lost by 3 days after application (Table 5). The effect on reproduction of BPH was also more obvious in immature adults than mature ones Adults treated with pymetrozine at their immature stage could not produce mature eggs (Table 6). From the results, it is considered that the reproduction of plant hoppers is suppressed due to reduction of oviposition as a result of delayed ovary maturation caused by feeding inhibition.

Table 5. Effect of pymetrozine topical application on oviposition of mature adults of BPH

Insecticides	Dose	No. of eggs (/ female / day)					
	µg / insect	1		2		3 DAA	
Pymetrozine	2.5	0	a	1.5	a	7.8	a
	0.25	3.2	b	7.7	b	7.0	a
Control	-	3.5	b	4.1	b	6.4	a

Means with differing letters are significantly different (P<0.05)

Table 6. Effect of pymetrozine topical application on fecundity of immature adults of BPH

Insecticides	Dose	No. of mature eggs / female			
	µg / insect	0-3		0-5 DAA	
Pymetrozine	2.5	0	a	1.5	a
	0.25	0	a	0.5	a
Control	-	4.3	b	17.0	b

Means with differing letters are significantly different (P<0.05)

Effect on walking speed of stink bug, *Eysarcoris lewisi*

No insecticidal activity was observed at a dose of 5ng/insect by topical application. Absence of diet did not affect the moving speed of stink bugs over the 72 hour period of study. However, pymetrozine paralysed their legs in the same way as observed in BPH and GRLH. Their moving speed was nearly half of control for the first 24 hours. However, the effect was reversible.

Treated insects recovered from paralysis by 48 hours after application (Table 7). The action is considered to be an important element explaining how pymetrozine reduces the dark spots on rice grains in the field.

Table 7. Effect on walking speed of *Eysarcoris lewisi* by topical application

Insecticides	Dose	Moving speed (mm / sec)									
	µg / insect	3		6		24		48		72 HAA	
Pymetrozine	5.0	16.5	a	15.3	a	11.6	a	18.5	a	24.0	a
	0.5	15.4	a	17.6	a	15.6	a	19.6	a	24.5	a
Diet absence	-	28.3	b	21.8	b	29.6	b	25.7	a	22.8	a
Control	-	29.7	b	27.7	b	22.7	b	20.4	a	29.0	a

Means with differing letters are significantly different (P<0.05)

Field trials

Nursery box application : *Sogatella furcifera* (WBPH)

Nursery box applications of pymetrozine suppressed WBPH populations for long periods as shown in Table 8. The first generation migrated into the site at about 40 days after transplanting (DAT) and the following generations peaked at 56 and 84 DAT in the control plots. On the other hand, the population density was suppressed and remained very low in the pymetrozine-treated plots until 96DAT. The effect was equal to that of imidacloprid. From the results, nursery box application at the rate of 300 g a.i./ha of pymetrozine Gr3 will provide nearly season-long suppression of *S. furucifera*.

Table 8. Control of *Sogatella furcifera* by pymetrozine in nursery box application (Kanagawa, Japan, 1995)

Insecticides	Dose	No. of insects (/ 100 hills)								
	g a.i./ha	42	49	56	63	70	77	84	89	96 DAT
Pymetrozine G3	300	0a	10a	56a	44a	2a	7a	24a	41a	21a
Imidacloprid G2	200	0a	10a	84b	44a	16b	5a	29a	67a	25a
Control	-	2a	15a	196c	72b	60c	36b	317b	108b	47b

(Means with differing letters are significantly different (P<0.05))

Foliar application against *Sogatella furucifera* (WBPH)

Foliar application of either the wettable powder (WP) or dust formulation of pymetrozine suppressed WBPH populations for a month as shown in Table 9. Pymetrozine was applied when populations reached a high density. WBPH populations decreased rapidly after application and were kept quite low for a month. The practical use rates of pymetrozine by foliar application were 63-125 g a.i./ha as WP25 and 200 g a.i./ha as 0.5% dust. These doses as different formulations provided comparable as good efficacy to buprofezine dust 1.5% at 600 g a.i./ha.

Table 9. Control of pymetrozine on *Sogatella furcifera* by foliar application (Kanagawa, Japan, 1995)

Insecticides	Dose	No. of insects (/90 hills)						
	g a.i./ha	0	2	7	14	21	28	35 DAA
Pymetrozine Dust 0.5	200	310a	6a	3a	3a	0a	15a	35a
Pymetrozine WP25	125	325a	49b	12b	5a	2a	10a	43a
Pymetrozine WP25	63	379a	28b	15b	2a	4a	21a	50a
Buprofezine Dust 1.5	600	418a	28b	10b	4a	4a	15a	46a
Control	-	417a	188c	119c	20b	17b	125b	127b

(Means with differing letters are significantly different ($P<0.05$)

CONCLUSIONS

Pymetrozine showed interesting modes of action against homopterous insect pests in paddy rice including knock-down activity induced by leg paralysis (particularly through foliar application), feeding inhibition and reduced fecundity. Although pymetrozine does not provide good insecticidal activity under laboratory conditions, these activity patterns are considered to work together in the field and provide its stable and long-lasting field performance. Together with its excellent environmental and human safety aspects, pymetrozine will be a useful element for sucking-pest control in paddy rice. Its high selectivity may be a useful feature for IPM programmes.

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EFFECTS OF LAMBDA-CYHALOTHRIN ON NATURAL ENEMIES OF RICE INSECT PESTS

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ABSTRACT

The effects of lambda-cyhalothrin applications on natural enemies of rice insect pests were investigated during the wet season of 1994 in central Luzon, Philippines. The primary objective of the study was to evaluate the potential for using lambda-cyhalothrin within integrated pest management (IPM) programmes in paddy-rice systems. Assessments were made on large plots (>1000m^2) for conservation of natural enemy populations, degree of pest control, cost effectiveness and yield production. Non-metric multidimensional scaling ordination was used to analyse changes in the community structure of predator and pest populations. Applications of lambda-cyhalothrin resulted in a limited reduction in the total number of natural enemy populations immediately after treatment, but numbers rapidly recovered within 15 days post-treatment. In all lambda-cyhalothrin treated plots the predator to pest ratio remained similar to control plots, therefore maintaining beneficial capacity. Treatments had very little effect on the relative proportion of important natural enemy groups or on individual species composition, throughout the season. On a cost-benefit analysis, the small investment in lambda-cyhalothrin provided substantial return to the farmer. It is concluded that lambda-cyhalothrin can be used within IPM programmes in rice agriculture, provided farmers are made fully aware of correct use patterns for maximum economic benefits and minimum environmental impact.

INTRODUCTION

The pyrethroid insecticide lambda-cyhalothrin (Karate 2.5% EC) is highly efficacious towards the major rice pests including green leafhopper, armyworm, rice bug and leaffolder. With integrated rice-fish culture becoming increasingly common in Asia, it is important to understand effects of lambda-cyhalothrin within this farming approach and thus make suitable use recommendations to benefit the farmer. Extensive aquatic field studies have repeatedly demonstrated the negligible risk of lambda-cyhalothrin use in systems where fish may be exposed (Hill, 1985). Detailed knowledge of effects on natural enemies of rice pests is however lacking. To explore the possibility of lambda-cyhalothrin being used within an IPM programme in tropical rice farming, the present large scale field study was undertaken with the following aims:

i) To measure the magnitude and duration of effects of lambda-cyhalothrin on key natural enemies in rice in the Philippines, and monitor subsequent recovery after treatment.

ii) To determine if treatments cause late-season resurgence of pest populations.

iii) To determine if any impact on natural enemy and pest populations translates into yield reductions.

MATERIALS AND METHODS

A 2.5 ha rice paddy farm near Cabanatuan City in Nueva Ecija, Luzon, Philippines, was chosen to conduct the trial, in collaboration with the Philippines Rice Research Institute (PhilRice). Twenty paddy fields of approximately equal size (>1000m^2) were separated into 4 replicate blocks of 5 plots. The brown planthopper (BPH) resistant variety IR64 was used, being representative of the most common variety planted in the Philippines and some surrounding S E Asian countries. Irrigation and drainage canals were constructed to allow individual irrigation of plots and avoid interplot movement of insecticides. Different spray regimes of lambda-cyhalothrin were applied (Table 1) using a 16 litre knapsack sprayer, and compared to the reference compound monocrotophos (Azodrin EC).

Table 1. Treatment regimes

	Application details		
	Spray no.	DAT*	Rate (g a.i./ha)
Control	-	-	-
Monocrotophos	1	21	400
	2	43	400
	3	70	400
Lambda-cyhalothrin	-	-	-
(low input)	2	43	6.25
	3	70	6.25
Lambda-cyhalothrin	1	21	6.25
(medium input)	2	43	6.25
	3	70	6.25
Lambda-cyhalothrin	1	21	6.25
(high input)	2	43	9.0
	3	70	12.5

*Days after transplanting

A modified 'Farmcop' suction device and sweep nets were used for sampling natural enemies and pests. The abundance of arthropods was determined in samples taken every 7 days throughout the season until harvest. Final yield was determined from subplots placed randomly within each plot. These plots remained undisturbed, apart from pesticide applications, until harvest.

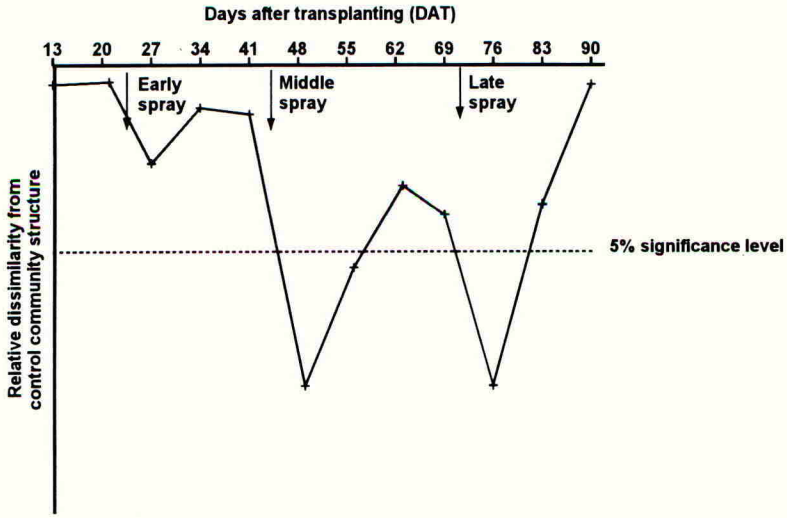
The PRIMER package (Clarke & Warwick, 1994) was used to statistically analyse the effects of the various lambda-cyhalothrin spray regimes on the predator and pest arthropod community structure. Data were analysed by multidimensional scaling ordination (MDS) using a square root transformation and the Bray-Curtis similarity index. The ANOSIM randomisation test (Clarke & Green, 1988) was used to determine statistical differences ($P<0.05$) in community structure between control and treated plots.

RESULTS

Effects of beneficial arthropods

Over 50 beneficial arthropod species were identified during the trial. Analysis of sweep net and suction sampling data showed there were 4 main groups of beneficials that were important in terms of abundance; araneae, coenagrionidae, coccinellidae and parasitoid wasps. In general, applications of lambda-cyhalothrin reduced the numbers of total natural enemies after treatment (Fig. 1), however population numbers were only significantly reduced ($P<0.05$, ANOSIM) compared to the control for between 10 and 15 days after application. Interestingly, the early season application of lambda-cyhalothrin did not significantly ($P<0.05$, ANOSIM) affect the beneficial community.

Figure 1. Relative dissimilarity between control and the highest lambda-cyhalothrin treatment regime of natural enemy populations. A significant change ($P<0.05$, ANOSIM) in community structure is represented below the horizontal dotted line.



Control of pests

Lambda-cyhalothrin showed good pest control (Fig. 2) particularly of green leafhopper (Fig. 3), and no resurgence problems. Other pests controlled included whorl maggot, rice leaf beetle, green semi-looper, cutworm, leaffolder, rice bug and whitebacked planthopper.

Figure 2. Relative dissimilarity between control and the highest lambda-cyhalothrin treatment regime of pest populations. A significant change ($P<0.05$, ANOSIM) in community structure is represented below the horizontal dotted line.

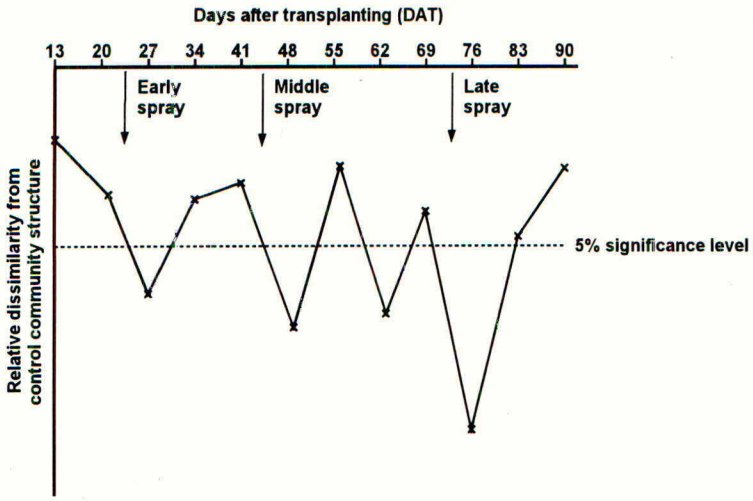
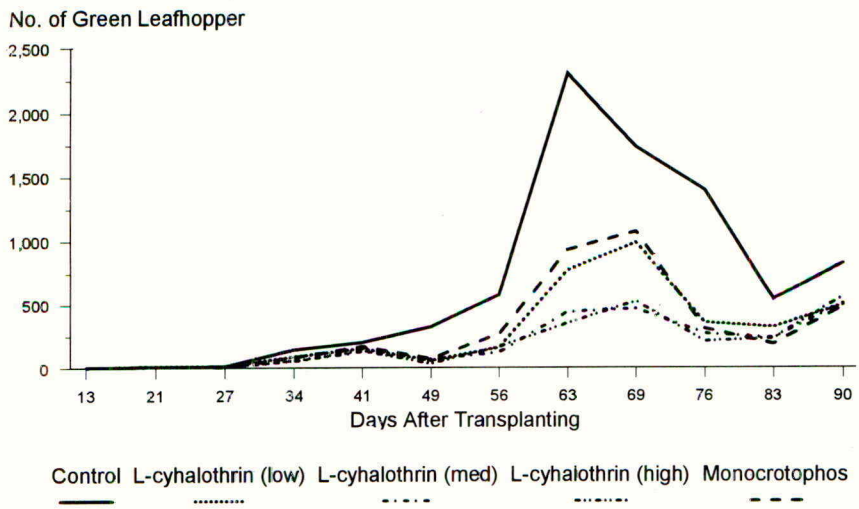


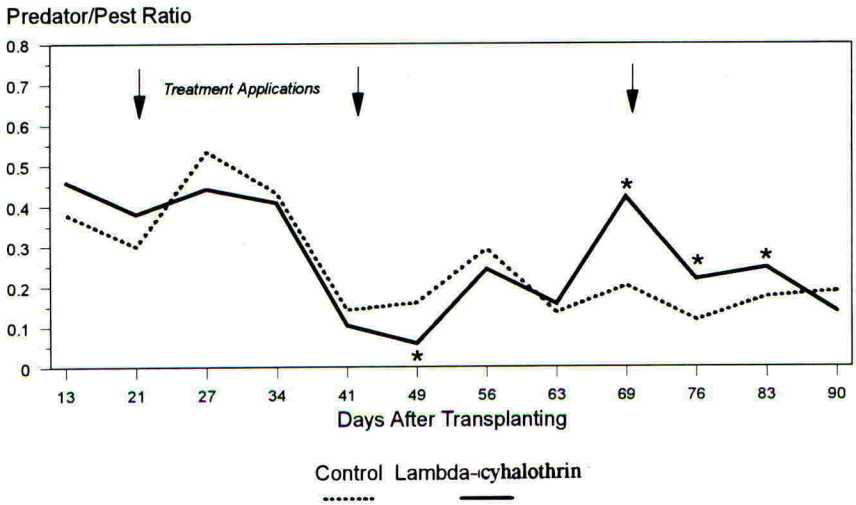
Figure 3. Mean abundance of green leafhopper under different spray regimes.



Predator/pest ratios

By comparing the ratio of natural enemies to pests in lambda-cyhalothrin treated plots with control data, it is possible to gain an understanding of any treatment-related disruption in the balance between beneficial and pest populations (Fig. 4). Throughout the season, lambda-cyhalothrin had little effect on the relative proportions of predators and pests within the experimental paddy fields, thus the beneficial capacity within the plots was not affected.

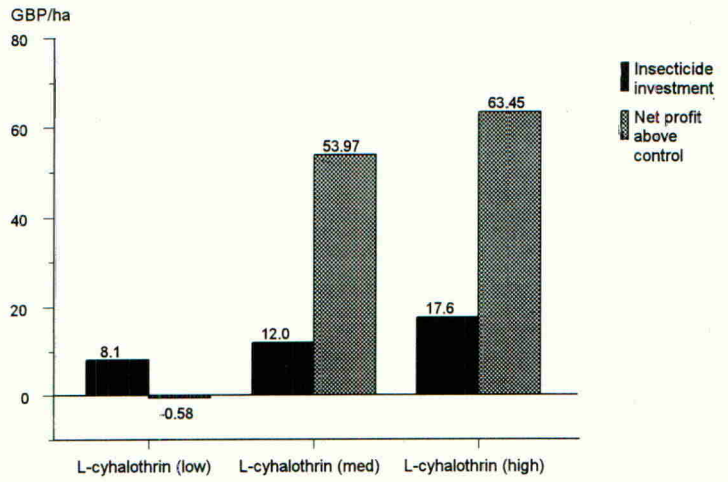
Figure 4. Comparison of predator to pest ratios between lambda-cyhalothrin (medium regime) and control plots (*significantly different to control, $P<0.05$ two-sided t-test).



Economics

Treatments of lambda-cyhalothrin, following the medium and high input regimes, significantly increased yield production ($P<0.05$, t-test) above the control. Maximum yield should not necessarily be the most important factor to farmers, a large proportion of the profit may have been spent on chemical control required to achieve this yield. In a cost-benefit analysis, the farmer need only make an extra 4% investment per hectare on the total input cost (seed, fertiliser etc.) for application of lambda-cyhalothrin following the high input regime. Even under the conditions of low pest pressure in this study, significant net profits above the control were achieved following the medium and high input lambda-cyhalothrin regimes (Fig. 5). However, avoiding the early season application did not result in an increased yield, indicating that the crop did not compensate for early season pest damage in this particular study.

Figure 5. Cost-benefit analysis for each treatment regime relative to the control.



DISCUSSION

Results indicate that lambda-cyhalothrin can be used within IPM programmes in rice agriculture, provided farmers are made fully aware of correct use patterns for maximum economic benefits and minimum environmental impact. On a hopper-resistant rice variety, 2 or 3 applications per season of lambda-cyhalothrin (Karate 2.5 EC) at 6.25 g a.i./ha will provide good cost-effective pest control under average pest pressure conditions, without causing resurgence problems. Following this use pattern, populations of natural enemies will be conserved thus providing additional control of pests.

ACKNOWLEDGEMENTS

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A PORTABLE MOTORISED AXIAL FAN AIR-ASSISTED CDA SPRAYER: A NEW APPROACH TO INSECT AND DISEASE CONTROL IN COFFEE

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ABSTRACT

A portable motorised air assisted CDA sprayer (the 'Motax') has been developed as the result of an extensive collaborative research project between the National Coffee Growers Federation of Colombia and the UK Overseas Development Administration, involving UK manufacturers Micron Sprayers Ltd. The objective of the project was to improve manual spray application methods in Colombian coffee for the control of coffee leaf rust. However, the sprayer is now also being considered for control of coffee berry borer, which has become a major problem in Colombia. The sprayer uses a rotary atomiser to produce uniformly-sized droplets which are propelled by a wide and turbulent air blast away from the operator, improving operator safety. Compared with traditional high volume techniques the sprayer offers the possibility of improved spray coverage and penetration at low total volumes of application, typically 30-70 litres/ha, allowing more timely spray treatments with the potential to adopt more responsive integrated insect pest and disease management strategies.

INTRODUCTION

Coffee leaf rust (*Hemileia vastatrix*) first reached Colombia in 1983 and was regarded as a major threat to Colombia's economy. Until then, Colombian coffee had been virtually free from insect pests and diseases, with no routine spraying of insecticides or fungicides. To combat the threat of coffee leaf rust, traditional high volume spraying techniques were initially introduced. These high volume techniques used pre-pressurised knapsack sprayers or semi-stationary pumps connected by long trailing hoses to lances fitted with hydraulic pressure nozzles. In both cases, total volume application rates of the commonly used copper-based fungicide formulations were in the range 200-500 litres/ha. Under Colombian conditions (dense planting of up to 10,000 plants/ha and steep slopes) the work rate was often as low as 0.2 ha/man/day, making the timely application of fungicides almost impossible.

It was against this background that a project was undertaken between the National Federation of Colombian Coffee Growers (FNCC) and the UK Overseas Development Administration (ODA), the objective of which was to develop appropriate portable low volume spraying equipment for coffee spraying in Colombia (Fernandez *et al.*, 1986). The programme involved UK consultants T.L.Wiles and Associates and spray equipment manufacturers Micron Sprayers Ltd.

Initially, the project examined both low volume techniques (30-50 litres/ha) and ultra-low volume techniques (5 litres/ha) combined with the use of electrostatics to improve underleaf coverage (Sharp *et al.*, 1986). All the prototype machines used rotary atomiser technology to achieve Controlled Droplet Application (CDA) i.e. narrow droplet size spectra.

The project investigated the distribution of spray droplets through the coffee bush and considered the effects of droplet size and number, formulation, air velocity and air-beam width and the influence of electrostatic charging of spray droplets. Of particular importance were the air-flow characteristics (Sharp *et al.*, 1988). Droplet distribution data from field trials and the results of bio-assay work allowed prototype machines to be assessed in terms of efficacy for disease control (Aston *et al.*, 1991).

Extensive field testing for efficacy against coffee leaf rust was undertaken with the prototype machine using a moderate velocity turbulent air-beam to carry the spray droplets into the crop foliage. This confirmed that the prototype machine was capable of applying sufficient quantities of copper (at reduced copper dosages compared to those used with traditional high volume sprayers) to control coffee leaf rust at a total application volume of only 50 litres/ha (Waller *et al.*, 1994). Operator contamination trials were undertaken during the project which clearly demonstrated the potential for improved operator safety with the low volume system.

During the project it became apparent that the threat from coffee leaf rust was not as severe as initially feared, particularly with the introduction of the resistant variety, 'Colombia'. However, coffee berry borer (*Hypothenemus hampei*), which poses a more significant and direct threat to coffee yields, was then reported in Colombia. The need to treat areas rapidly for control of coffee berry borer highlighted the need for application techniques with a high work rate and the potential use of insecticides by farmers against this insect pest meant that operator safety became a significant issue.

Further trials took place in the period 1992-1996, aimed at the continued development and validation of the machine, and confirmation of the acceptability of the spraying technique, in actual field operations. Micron Sprayers have redesigned the prototype machine in the light of field experience gained during the trials to make a production version, the 'Motax', and this paper describes these modifications.

DESCRIPTION OF THE MACHINE **(FIGURE 1)**

In addition to the key application criteria of atomiser design and air-flow characteristics the development of the sprayer has addressed the problems faced by spray operators working on steep (often slippery) slopes which require the operator to have one free hand for safety. The increased work rate possible with this sprayer has meant that it had to be designed to enable operators to use the sprayer over reasonably long periods of time without suffering discomfort. The critical design criteria considered were therefore the weight distribution, minimisation of vibration, and other ergonomic requirements e.g. position of controls.

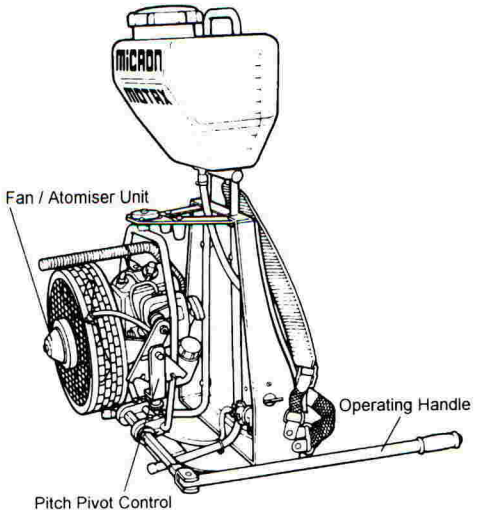
Mounting the sprayer on the operator's back ensures easy passage through the crop. As the centre of gravity is kept close to the operator, using wide shoulder straps and a waist band to

ensure good weight distribution, the sprayer can be carried without undue fatigue in normal operation. The other significant advantage of back mounting is operator safety as the operator is walking away from the spray emitted.

Deposition trials had shown that the best spray coverage was achieved by angling the air-beam into the row. It is possible to spray both sides of an inter-row with one spray pass by oscillating the air-beam from side to side. The engine/fan/atomiser unit is mounted in a light-weight cradle which is oscillated by a manually-operated handle. Throttle and spray flow valve controls are mounted on the sprayer and are easily accessible. The vertical (pitch) orientation of the engine/ fan/atomiser unit can be altered according to the crop and slope characteristics (without removing the sprayer from the operator's back).

For reasons of simplicity, spray liquid is fed to the rotary atomiser by gravity. The spray tank size has been selected to match the fuel tank capacity so that fuel and spray liquid will require refilling at the same time, thus minimising downtime or the possibility of the operator continuing to run the sprayer when the spray tank is empty.

Figure 1.Diagram of the production sprayer.



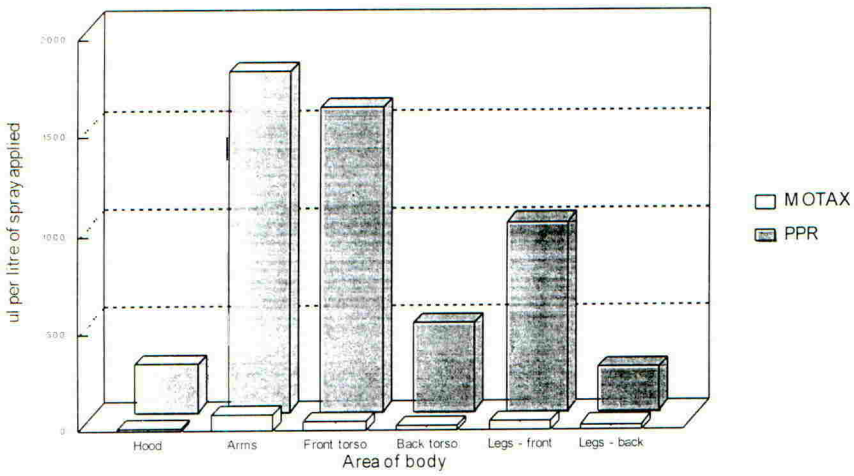
Specification of production sprayer

Weight: empty	10kg
ready to spray	18kg
Power source	33cc, 2 stroke engine
Fuel consumption	0.75 litres/h
Fan/Atomiser speed	6,500 rev/min
Airflow: speed	25m/s at fan
volume	0.8 m^3/s
throw	up to 8m
Flowrate range	50 - 300 ml/min
Droplet size range	80 - 120 μm VMD

OPERATOR SAFETY

Field trials to examine the level of operator contamination showed that, under a wide range of conditions, the air-assisted CDA sprayer greatly reduced levels of operator contamination compared with traditional high volume lever operated and pre-pressurised knapsack sprayers (PPRs) with a single hand lance (see Figure 2). It also became apparent that the majority of operator contamination from high volume applications occurred due to indirect transfer of spray as the operator walks through treated foliage. The backward-facing design of the air-assisted CDA sprayer ensures that the operator does not walk through treated foliage, thereby significantly reducing potential operator contamination.

Figure 2 Comparison of the potential dermal exposure to pesticides from alternative application techniques.



Apart from the inherent improvement in operator protection afforded by not walking through treated foliage, there are other specific safety features built into the sprayer. The tank filling aperture is of sufficiently large diameter to allow filling without spillage, the tank neck contains a filter which is deep and vented to suppress the tendency for splash back, and, very importantly, the tank cap has a seal and venting valve to prevent liquid leaking if the operator bends over. The sprayer also accommodates a safety 'cut out' switch in the end of the oscillation handle to stop the engine quickly if required.

BIOLOGICAL EFFICACY

Field trial results with the air-assisted CDA sprayer using copper oxychloride confirmed that at total application volumes of 50 litres/ha, using droplet sizes of around 100µm, good control of leaf rust was achieved - with good coverage of foliage, with laboratory trials showing that at 30 litres/ha the threshold for 100% inhibition of coffee leaf rust by copper oxychloride is 30-35 droplets/cm^2. Field trials were also undertaken comparing the sprayer with the standard PPR used in Colombia. Both machines applied copper oxychloride at a rate equivalent to 1.5 kg/ha, the air-assisted CDA sprayer at 50 l/ha and the PPR at 250 litres/ha, in coffee already heavily infected with leaf rust. Five applications of copper oxychloride were made at 30 day intervals and four applications at 45 day intervals. An untreated plot was included as the control. Results showed that the low volume treatments using the air-assisted CDA sprayer were at least as effective as the high volume PPR treatments (whether control was measured as the number of healthy leaves present or as a percentage of rusted leaves) and more effective at 30 day spray intervals (for which the higher work-rate

capability of the CDA sprayer is critical). A further trial demonstrated that the CDA sprayer was at least as effective as the PPR at preventing the further development of leaf rust at low disease levels **(Figures 3 and 4)**.

Figure 3. The effect of spray treatment on the proportion of leaves showing rust infection (from Waller et al. 1994)

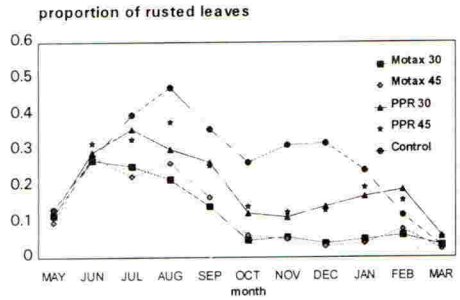
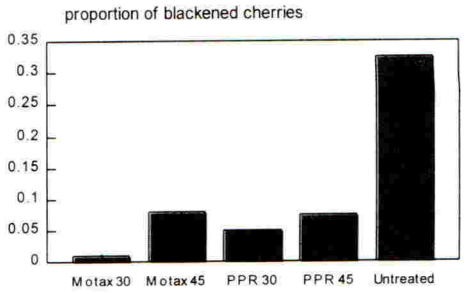


Figure 4. The effect of spray treatment on proportion of blackened coffee cherries (from Waller et al. 1994)



IMPLICATIONS OF LOW VOLUME APPLICATION METHODS FOR MANUAL SPRAY PROGRAMS

Low volume application with the air-assisted CDA sprayer offers the prospect of a five-fold improvement in work-rate compared with traditional high volume spraying techniques - from 0.2 ha/man/day to 1.0 ha/man/day or higher. Labour cost savings alone (based on the Colombian minimum wage) are over US$20 per hectare per application. For coffee leaf rust control, for which at least four spray treatments per year are required, the labour savings would be over US$80 per sprayed hectare. However, not only does a high work rate lead to reduced application costs but it can ensure sprays are applied at the correct time - thus allowing usage of agrochemicals to be minimised (with substantial consequential cost savings). The ability to spray rapidly may be of critical importance in coffee berry borer control programmes, where large areas need to be treated quickly and many insecticides show no significant residual effect after 15 days (Villalba *et al.*, 1995). Minimisation of agrochemical usage and improved spray targeting are pre-requisites for the use of agrochemicals within Integrated Pest Management (IPM) programmes.

CONCLUSIONS

The 'Motax' is the result of an extensive research project on optimising manual spray application in Colombian coffee. This portable air-assisted CDA sprayer offers the significant benefits of increased work rate, improved spray penetration and coverage and potential reductions in operator and environmental contamination compared with traditional high volume techniques. It offers the prospect of implementing IPM programmes with reduced use of pesticides, better spray targeting, improved operator safety and less off-target environmental contamination.

The sprayer has undergone extensive field validation trials, and has now been production-engineered to offer a robust and practical tool for farmers. Although commercialisation of the sprayer is being undertaken for coffee in Colombia, the air-flow and operator safety characteristics inherent in its design offer an advanced low volume alternative to the traditional high volume techniques currently used world-wide on many bush and vine crops.

ACKNOWLEDGEMENTS

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POST-HARVEST TREATMENT OF BANANAS IN THE WINDWARD ISLANDS

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ABSTRACT

Bananas grown in the Windward Islands are affected by crown rot, a fungal disease controlled by the post-harvest application of the fungicides imazalil and thiabendazole. Proper management of these fungicides, used by many thousands of small-holder farmers, is essential in order to maintain their effectiveness, to satisfy international marketing requirements and to protect the users and the environment.

INTRODUCTION

The effective management and control of pests and diseases in the tropics can be extremely difficult; pest and disease populations can be high and the prevailing climate encourages their rapid development and spread. Where crop production is at the estate level, resources for crop protection are generally adequate with well-organised schedules of crop protection measures. However, where production is in the hands of small-holder farmers, effective pest control becomes much more difficult with scope for error and abuse of recommended practices. These problems become compounded for those producing countries which have only limited resources to devote to agricultural extension services and where some growers receive insufficient guidance and others choose to ignore marketing requirements.

The Windward Islands, in the Caribbean, are major banana producers relying mainly on small-holder farmers; across the four producing Islands (Dominica, Grenada, St Lucia and St Vincent) there are about 20,000 registered growers. Fruit from the Islands is affected by crown rot, a disease caused by a mix of fungal pathogens of which *Colletotrichum* spp. and *Fusarium* spp. are the most significant. The disease causes darkening of the banana fruit stalk tissue (pedicel), often accompanied by the presence of a surface mould. In severe cases, the banana fingers can separate from the hand, or cluster, and the pulp of the banana becomes soft and inedible. The fruit is unacceptable to the consumer resulting in downgrading or rejection, with substantial financial implications for the producers.

The level of crown rot fluctuates at the height of the crown rot season (September - December) with some 5 -10% of shipped fruit being affected. The disease is controlled by post-harvest immersion of the cut fruit, for thirty seconds, in a fungicidal solution or suspension at an active ingredient concentration of 0.05% (500µg/ml). The fungicide is applied by the grower, at field packing stations immediately before the fruit is boxed for shipment. In any one week, 3000 to 5000 growers may be individually involved in fungicide application and there is considerable scope for error and mis-management.

The use of fungicides in such situations requires active management to ensure that the treatments are, and remain, effective and with due regard to the safety of the operator, the consumer and to the environment.

PURPOSE OF THE STUDY

The effectiveness of crown rot control and the proper management of the fungicides used for this purpose depend on a number of factors:

(i) the identification of the fungal pathogens causing the disease, the determination of their tolerance to existing fungicides and the selection of the most appropriate permitted fungicide;
(ii) proper treatment of the fruit with the appropriate fungicide at the effective active ingredient concentration;
(iii) providing means for extension workers and quality inspectors to check that the fruit has been treated and also that the treatment solutions are being prepared at the recommended levels;
(iv) ensuring the safety of those involved in the dipping process,
(v) developing means for allowing the safe disposal of waste and used banana dip liquors.

These factors cannot be treated in isolation and all were incorporated within the defined objectives of the project. In addition, effective dissemination of the findings was considered to be crucial to the success of the project.

RESULTS

Isolation of crown rot fungal pathogens and determination of fungicide tolerance levels

Isolates of cultures grown from tissue samples from fungicide-treated, commercially shipped, crown-rot infected fruit showed the crown rot pathogen mix to be similar to that identified in earlier studies (Johanson and Blasquez, 1989). The isolated fungi were mainly from four genera: *Fusarium*, *Colletotrichum*, *Botryodiplodia* and *Pestalotia*. In addition *Penicillium*, *Nigrospora*, *Phoma*, *Diplodia*, *Dactylella* and *Trichoderma* were isolated infrequently. *Colletotrichum* spp. and *Fusarium* spp. were the most common and occurred with equal frequency in fruit from St Lucia and St Vincent and with both control methods in use at that time (dipping and crown pads).

Colletotrichum spp. and *Fusarium* spp. isolates had their ED_{50} s (Effective Dose required to kill/reduce growth of 50% of the population) in the same range as those tested by Johanson and Blasquez. *Colletotrichum* spp. had an ED_{50} range of 1.2-2.4µg/ml, while Johanson and Blasquez reported values of 1.48 and 1.58µg/ml. *Fusarium* spp. recorded a range of 0.10-0.3µg/ml compared to 0.04-0.4µg/ml in 1989. This indicates that there is no build up of insensitivity in these fungi. The secondary pathogens *Botryodiplodia* spp. and *Pestalotia* spp. had ED_{50} s in the ranges 0.11-0.24µg/ml and 0.14-0.3µg/ml respectively. The results for *Botryodiplodia* spp. isolates are similar to the ED_{50} of 0.19µg/ml reported earlier by Johanson and Blasquez.

From the data there appears not to have been a substantial change in the populations of fungi associated with the Crown rot complex in the islands of St. Lucia or St. Vincent. The screening of fungi for insensitivity to Imazalil shows no sign of any build up of tolerance to the fungicide. The high occurrence of bacteria in the first isolations forced the introduction of antibiotics to the media. Though bacteria were recorded in the 1989 study, an antibiotic was not required. This earlier work indicated that bacteria have no place in the initiation of crown rot. However, the high levels of bacteria encountered in this recent study may warrant further investigation since it has been reported that some bacteria have a synergistic relationship with fungi (Lukezic and Kaiser, 1966).

Apart from thiabendazole, which has previously been used for crown rot control, there are no other suitable compounds presently available which are as effective, or which could replace, imazalil for crown rot control. The use of these two compounds must thus be carefully managed to preserve their effectiveness and particularly to guard against tolerance development. Imazalil is a triazole compound and another triazole, propiconazole, is used for the control of banana leaf spot. To reduce the risk of any cross-tolerance developing, thiabendazole should be used during the low season for crown rot (January - May) with imazalil being used for the remainder of the year. Management in this way will reduce the risk of tolerance development.

Fungicide selection and management

The scope for the evaluation of alternative fungicides for use in the banana industry is limited; currently there appear to be no suitable candidate compounds for crown rot control although there may be scope for the further modification of existing, or the development of new, formulations of the preferred active ingredients, thiabendazole and imazalil, to improve their activity. Minor modifications can be attempted locally, in consultation with the suppliers e.g. examining the effects of pH change in the dip liquor However, the manufacturers of the products will, themselves, need to conduct any further studies aimed at significantly modifying the formulation, to ensure that the changes do not result in the need to develop substantial additional registration data for the product. Further development by the manufacturers is, however, dependant upon the overall volume of sales and an assessment of the economic return. In a limited market, further development may not be justified.

Within the constraints of the banana production system in the Windward Islands, unique in that the production lies in the hands of many thousands of small growers, rather than a large-scale plantation produced crop, dipping is probably the most appropriate system for effective fungicide application. As such, there is no scope for any reduction in the overall quantities of fungicide presently used by growers. For latent fruit infections, the use of fungicidal stem injection may be a complementary treatment to dipping and there is scope for evaluation of a simple, hand-held foam applicator for banana crown treatment.

However,the practice of dipping creates a significant likelihood of operator contamination. Most workers dip the produce using bare hands to immerse and remove the fruit, and exposure times for some workers (generally women) could be between 3 and 4 hours per working day, perhaps more on some larger farms. Fungicidal suspension also runs off onto the clothes of those doing the dipping, allowing for continued exposure after the dipping process has been completed. Field hygiene

practices are also poor and the risk of ingestion of residues from eating/smoking without previously washing or changing clothes is high; babies and small children, fed by their mothers in the field, are also open to the risk of exposure. Lax control of dipping practices with pesticidal products also allows the development of bad habits which may be more significant if more toxic products are brought into use. Appropriate, low-cost, gloves and aprons must be made available for use by these workers; many already appreciate the potential hazard and the rate of uptake is likely to be good as long as costs can be kept low and the gloves, particularly, are considered suitable (flexible, tight fitting and elbow length). Those involved in packing the treated fruit are also at risk; white deposits were observed on the hands of those involved in this work and they too should be encouraged to wear gloves. A major education programme will be needed to emphasise to growers the potential hazards of current practice.

Fungicide waste disposal

Current fungicide disposal practice is for waste fungicide solution to be tipped onto the ground or into shallow soil pits. An initial assessment of fungicide mobility using soil columns, showed that despite Windward Island soils showing some, but variable, capacity for absorbing the fungicide imazalil, there is the potential for significant leaching to occur, particularly so with the lighter, sandier soils of St Vincent. The analysis of soil depth profile samples from used soil disposal pits in St Lucia confirmed the expected penetration of residues and thus confirmed the need for improved procedures for the safe and effective disposal of fungicide waste.

Imazalil, the currently recommended fungicide for post-harvest use in the Windward Islands is chemically stable except under quite strong conditions, and attempts to decompose the fungicide in aqueous solution using low-cost, common, locally available chemicals proved, as expected, unsuccessful. Attention was thus focused on adapting the current disposal pit system to minimise the quantities of fungicide entering the soil and becoming available for leaching. Laboratory trials using charcoal proved the most effective filtration medium and field trials were established in St Lucia. Lining the base of the pit with charcoal proved reasonably effective in adsorbing the fungicide and retaining the material for further treatment by burning or by allowing natural degradation to occur. This work and subsequent studies on the additional use of banana trash within the disposal pit, has allowed recommendations to be made for an improved disposal pit, which is also suitable for use with thiabendazole.

Test procedure for determining whether fruit has been treated with fungicide

A procedure, using the NRI thiabendazole/imazalil test kit and based on the reaction of the fungicides with a buffered bromocresol green reagent, was developed and evaluated for its effectivness in determining whether banana clusters had been treated with fungicide (Cox and Kilminster, 1993). This procedure complements the existing procedure for monitoring fungicide concentrations in banana dip liquors and enables produce quality inspectors to ensure that bananas for export are being treated with fungicide, as required by the Banana Growers Association (BGA) and that crown rot incidence is not due to non-treatment on the part of growers.

The test was introduced by St Lucia in October 1995 to examine samples of fruit brought by growers to the shipping wharf at Castries. BGA policy was to reject fruit

found to be untreated with fungicide and initially high levels of non-treatment were detected. The test system received widespread publicity and non-treatment cases were seen to steadily drop, associated with a substantial decrease in the measured level of crown rot in exported fruit. The test was also introduced at the Vieux Fort wharf, St Lucia and at the Inland Buying Depots used by growers producing only small quantities of fruit or those unable to take their fruit to the wharf. Dominica introduced the test system in March 1996 and Grenada and St Vincent also intend to introduce the system.

The BGAs are pleased with the initial success of the testing procedure and the pressure that this has put on growers to improve their fungicide application practices. Fruit quality has improved although it is too early to calculate the financial benefits

Dissemination

Crown rot control and fungicide management workshops were held in Dominica, St Lucia and St Vincent (Cox, 1996) for fruit quality and extension officers and for selected growers. Two workshops were held on each island, one workshop for each of the two audience groups, with the presentations being targeted at the requirements of the different groups. These workshops enabled the Windward Islands Banana Development and Exporting Company (WIBDECO) to further their education programme and to stress the importance of good management in the banana industry.

The workshops were an effective dissemination medium and attracted much interest. In each of the Islands, sound or video recordings were made of the presentations and these were used in radio and television news broadcasts and in specific scheduled programmes for banana growers. Some of the material is also being used by the Island BGAs, in liaison with WIBDECO, for the production of specific training aids, including videos.

Additionally, reports on each element of this study were copied to managers and extension workers of the Island BGAs through WIBDECO for broader dissemination.

SUMMARY

This study allowed a thorough review of fungicide management practices within the Windward Islands banana industry and of the scope for their improvement within marketing constraints, the limitations of international pesticide legislation and guidance and the logistical and financial limitations on growers. The project was timely, with the outputs being delivered at a time of great pressure on the industry to improve fruit quality and to develop a greater environmental awareness. Workshops for growers were considered to be a valuable contribution towards improved communication between the growers and the extension services. Considered individually, the outputs are small. However, when considered as a package to support a fragile industry, the outputs underpin key areas of activity. They will be beneficial to the industry and should play a significant role in maintaining the acceptability of the Islands as producers for the European Union (EU), and particularly with increased concerns for the global environment. The developments on fungicide waste disposal, particularly, make a further, positive, step towards responsible pesticide management.

The use of the fungicide test kit, by enabling the enforcement of a strict policy of rejecting untreated fruit, has forced growers to comply with BGA requirements for fungicide treatment and this has helped to contribute to a reduction in the observed levels of banana crown rot. Results from the first six months of operation of the testing programme in St Lucia, shows a substantial reduction in the number of cases of non-treatment detected, and a corresponding reduction in the incidence of crown rot in shipped fruit. The procedure thus acts as a deterrent to those trying to avoid the cost of fungicide use whilst, more constructively, it can also be used as a diagnostic tool to help extension workers/growers to identify causes of poor treatment, monitor for excessive levels of residues etc.

FOLLOW - UP

The ultimate success of the project in the Windward Islands will depend upon continued inputs from WIBDECO to maintain the momentum generated by the workshops and to ensure that all the components of the management practice are implemented. In particular, the introduction of the fungicide disposal pits will undoubtedly prove difficult. It is essential for the future of the industry that such measures are introduced, however, and growers need to be persuaded of their importance. Inclusion of satisfactory disposal systems as a measure in the field assessment of individual growers may be needed to further this process.

To determine the degree of success of the project and to address any difficulties that have arisen, a follow-up visit is intended in early 1997, also enabling a further series of workshops to be held.

ACKNOWLEDGEMENTS

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EFFICACY OF PRODUCTS DERIVED FROM INDIGENOUS PLANTS FOR THE CONTROL OF THE LARGER GRAIN BORER (*PROSTEPHANUS TRUNCATUS*)

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ABSTRACT

The recent introduction and establishment of the larger grain borer (*Prostephanus truncatus*) into Africa has greatly increased pest problems in maize storage. The use of locally-available plant materials to limit insect damage in stored foodstuffs is a common practice in traditional farm storage in developing countries. *Ocimum* species grow widely in many parts of E & S Africa and some local farmers mix stored foodstuffs with dry leaves of these plants for protection against insect pest damage. The scientific basis for this practice was investigated by evaluating the biological activity of its major essential oil components alone, and in combination with sunflower, sesame, mustard and coconut oils, against *P. truncatus*. Eugenol and 1,8 cineole were identified as the major essential oils in the leaves of *O. suave* and *O. kenyense,* respectively. Each compound, applied topically or impregnated into maize grains, was highly toxic to the beetles, inducing 100% mortalities within 24 h. Development of eggs and immature stages within grain kernels, as well as progeny emergence, were completely inhibited in treated grain. Eugenol was also highly repellent to *P. truncatus*. When each compound was applied alone, there was a highly significant loss of activity within 24 hours. Combining the compounds with other plant oils maintained their activity and provided protection of maize for 90 days.

INTRODUCTION

Insect pest damage to stored grain results in extensive losses to farmers throughout the developing world. Recently, a major storage beetle pest, the larger grain borer (*P. truncatus*) (Bostrichidae), native to Central America, has become established in East and West Africa and is causing significant problems through its ability to damage dried maize (Dick, 1988). Many small-scale farmers in Africa commonly mix stored grain with different kinds of plant products for protection against pest damage in storage (Hassanali *et al.,* 1990; Poswal & Akpa, 1991). *Ocimum suave* and *O. kenyense* (Labiatae) are aromatic shrubs widely grown in many parts of Africa and Asia (Paton, 1991). They have traditionally been used as insect repellents, particularly against mosquitoes (Kokwaro, 1976). Some local farmers also mix stored foodstuffs with dry leaves of these plants to protect them against insect infestation (Hassanali *et al.*, 1990). More recently, ground leaves and essential oil extract of *O.*

kenyense were shown in laboratory bioassays to be effective protectants of maize and sorghum against attack by several storage beetles (Bekele, 1994; Jembere *et al.*, 1994).
The efficacy of plant oils as grain protectants has been tested against several insects (Obeng-Ofori, 1995) and Don-Pedro (1989) suggested the possibility of using oils in combination with synthetic insecticides in simple mixtures as a means of making their use more effective. The work reported here employs this approach by investigating the bioactivity of plant oils and essential oil components alone and combined in simple mixtures, for protection of stored maize against damage by *P. truncatus*.

MATERIALS AND METHODS

Chemical composition of essential oil

Essential oils were extracted from the leaves, inflorescences and green stems of *O. suave* and *O. kenyense* by steam distillation and analysed using GC-MS (VG12-250 equipped with data system) and gas chromatography (Hewlett Packard 5790). The constituent compounds were identified by spectral comparison with synthetic standards. Eugenol and 1,8 cineole were the major components, comprising 60% and 40% of the total collection from *O. suave* and *O. kenyense*, respectively (Jembere *et al.*, 1994).

Contact toxicity by topical application

P. truncatus obtained from a laboratory stock culture maintained at the Institute for Stored Product Protection, Berlin, was reared in a controlled environment room at 27±1 °C and 65-70% r.h. in the dark on whole maize grains. The standard toxicity method described by McDonald *et al.* (1970) was used. 2 µl of different doses of test solution were applied to the dorsal surface of the thorax of each insect with a micro-applicator (50 beetles in five replicates of 10 per dose). Similar numbers were treated with solvent only as control. After treatment, insects were transferred into 11 cm diameter glass Petri dishes (10 insects/Petri dish) containing food. Insect mortalities were recorded 48 h after treatment.

Toxicity and persistence in grain

500 g samples of maize were mixed with test solution at 0, 0.5, 1, 5 or 10 µl/kg for 10 minutes to ensure even distribution of the solutions. Solvent was then allowed to evaporate completely. 20 beetles were exposed to treated grain which had been stored for 1, 10, 20 or 40 days. Mortality was assessed after 24 h exposure. To test effects against hidden eggs and immature stages, batches of 200 g of maize in 300 ml glass jars were infested with 50 adults (3-10 days old) to allow egg laying. The parent adults were removed after seven days and 1 day later, 4 batches of the grain were treated with each chemical at 0.5, 1 or 5 µl/kg. Thereafter, these treatments were repeated one, two and three weeks after adult removal. Adults subsequently emerging were counted for a period of 8 weeks following the removal of adults.

Repellency bioassays

Test arenas consisted of 11 cm Whatman No. 1 filter papers cut in half. Each solution was applied to a half filter paper disc as uniformly as possible with a pipette. The other filter paper halves were treated with acetone alone. Full discs were then remade by taping treated halves to untreated halves of the same dimensions. Each filter paper was placed in a petri dish and 10 beetles of mixed sex were released at the centre of each filter paper disc and then covered. Each treatment was replicated 10 times. The number of insects present on control (N_C) and treated (N_T) strip were recorded after 1 h exposure. Percent repellency (PR) values were computed as $PR = [(N_C-N_T/(N_c+N_T)] \times 100$.

Toxicity bioassays with essential oil and plant oil mixtures

Coconut oil (locally extracted in Ghana) and refined oils (sunflower, sesame, mustard) were used. Different doses, 0.5, 1 or 5 µl of each compound per kg of maize were mixed with different oils (coconut, sunflower, sesame or mustard) at the rate of 5 ml/kg. Samples of 500g of maize were mixed with test solutions by tumbling in glass jars. The effect of the oils alone and in combination with the chemicals on adult mortality was bioassayed: 20 (1-week-old) adults of mixed sex were exposed to treated grain which had been stored for 10 to 90 days. Mortality was assessed in 5 replicate jars after 24 h exposure.

RESULTS

Contact toxicity, toxicity and persistence in grain and repellency

Eugenol or 1,8 cineole applied topically was highly toxic to *P. truncatus* and toxicity was dose-dependent (Table 1a). All treatments containing eugenol or 1,8 cineole killed all the beetles exposed within 24 h, irrespective of dosage (Table 1b). There was, however, a highly significant loss of toxicity after only 24 h following treatment. Each compound completely inhibited the development of eggs, larvae and pupae of *P. truncatus* hidden inside maize kernels. When grain containing eggs, first and second larval instars or pupae were treated with each compound, no progeny emerged after 8 weeks. Eugenol evoked strong repellency in the beetle but 1,8 cineole was moderately repellent. (Table 1c). Repellency was also dose-dependent.

Table 1a. Toxicity of 1,8 cineole and eugenol applied topically to *P. truncatus*

Dosage	Percent adult mortality after 48 h	
(µl/beetle)	1,8 cineole	Eugenol
40	0^{e}	0
1	45^{d}	50
3	75^{c}	87
5	91^{ab}	93
7	100^{a}	100^{a}
10	100^{a}	100^{a}

Table 1b. Mortality (%) of *P. truncatus* in treated maize at different storage intervals

Dosage (µl/kg)	Time after treatment				
	3h	1d	10d	20d	40d
1,8 cineole					
0	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
1	100^{a}	20^{c}	5^{d}	1^{d}	0^{d}
5	100^{a}	32^{b}	5^{d}	0^{d}	0^{d}
10	100^{a}	39^{b}	5^{d}	1^{d}	0^{d}
Eugenol					
0	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
1	100 ^{a}	23^{c}	7^{d}	2^{d}	0^{d}
5	100^{a}	36^{b}	16^{c}	2^{d}	0^{d}
10	100^{a}	41^{b}	20^{c}	3^{d}	0^{d}

Table 1c. Percent Repellency of 1,8 cineole and eugenol against *P. truncatus*

Dosage (µl/disc)	Mean percent repellency (PR)	
	1,8 cineole	Eugenol
0.5	14^{f}	49^{d}
1.0	18^{f}	64^{c}
3.0	30^{e}	80^{b}
5.0	43de	86^{b}
10.0	50^{d}	94^{a}

Means followed by different letters are significantly different at the 0.05 level, DMRT

Toxicity of mixtures

All treatments comprising plant oils alone or combined with either 1,8 cineole or eugenol caused significant ($P<0.001$) mortality compared to untreated grain or grains treated with essential oils only (Table 2). Plant oils alone were less effective against the beetle than when combined with an essential oil. Mortality significantly ($P<0.001$) decreased with time after application except treatments combining plant oils and essential oil compounds which achieved 100% mortality after 90 days storage following application.

Table 2. Percent adult mortality of *P. truncatus* in plant oil/eugenol or 1,8 cineole treated maize after different intervals of storage

Treatments	Days of storage after treatment			
	10	30	60	90
Control	0[d]	0[d]	0[d]	0[d]
1,8 cineole (0.5 µl/kg)	1[d]	0[d]	0[d]	0[d]
1,8 cineole (1.0 µl/kg)	2[d]	0[d]	0[d]	0[d]
1,8 cineole (5.0 µl/kg)	3[d]	0[d]	0[d]	0[d]
Eugenol (0.5 µl/kg)	0[d]	0[d]	0[d]	0[d]
Eugenol (1.0 µl/kg)	5[d]	0[d]	0[d]	0[d]
Eugenol (5.0 µl/kg)	20[c]	4[f]	0[d]	0[d]
Coconut oil at 5 ml/kg	41[b]	13[e]	2[d]	0[d]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]
Sunflower oil at 5 ml/kg)	23[c]	3[d]	0[d]	0[d]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]
Sesame oil at 5 ml/kg	35[b]	10[e]	2[d]	0[d]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]
Mustard oil at 5 ml/kg	39[b]	12[e]	1[d]	0[d]
+ 1,8 cineole	100[a]	100[a]	100[a]	100[a]
+ eugenol	100[a]	100[a]	100[a]	100[a]

Mean of 5 replicate assays. Values followed by different letter(s) are significantly different at the 5% level, DMRT

DISCUSSION

In this study, eugenol and 1,8 cineole applied topically or impregnated on whole maize grains were highly toxic to *P. truncatus*. Ryan and Byrne (1988) attributed the toxicity of terpenoids including linalool and 1,8 cineole against *Tribolium castaneum* to their reversible competitive inhibition of acetylcholinesterase. Eugenol evoked strong repellent action against the beetle. Both compounds completely inhibited the development of eggs and immature stages within grain kernels, thus increasing their protectant potential against insect damage in storage. The efficacy of *O. suave* and *O. kenyense* in protecting foodstuffs against insect damage in traditional grain stores in East Africa (Bekele, 1994) may be attributed to the high concentrations of eugenol and 1,8 cineole in the leaves. The enhanced toxicity and persistence of 1,8 cineole and eugenol when combined with plant oils may be due to a reduced rate of evaporation of the compounds and increased uniformity of distribution over the grain surface, thereby increasing contact with the insects. Oils can slow the rate of penetration of chemicals into plant interiors (e.g. grains) and increase the rate of insecticide penetration into insect cuticle (Anderson *et al.*, 1986), which may increase the probability of an insect contacting a lethal dose of the chemicals.

Our results support previous findings that a wide range of monoterpenes have insecticidal and suppressive effects on the reproductive development of several insect species (Jembere *et al.,* 1994). *P. truncatus* is the most destructive pest of stored maize and cassava in sub-Saharan Africa. The exploitation of locally available plant materials to suplement the use of synthetic insecticides would increase the cost-effectiveness of its control in the new environment in Africa. Future work will focus on understanding the mode of action of these compounds, particularly regarding their penetration through insect cuticle and grain testa and their effects on mammals fed on treated food. Detailed toxicological studies are required before they could be recommended for use in stored product protection.

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SUCCESS OF *TRICHOGRAMMA CHILONIS* (ISHII) FOR AREA-WIDE CONTROL OF SUGARCANE BORERS IN PAKISTAN

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ABSTRACT

Large scale control of sugarcane borers was achieved in Al-Noor Sugar Mills area by inundative releases of *Trichogramma chilonis*. The treatment of 7,100, 27,000 and 30,000 acres of sugarcane with *T.chilonis* during 1992-95 seasons checked the borer infestation below economic threshold level. The parasite recovery from field collected egg masses of sugarcane borers ranged from 43-68% from April to September, which indicated the establishment of parasitoids in the target area. These studies manifest the effectiveness of this parasitoid in controlling the sugarcane borers and its potential for inclusion in integrated pest management for area-wide control programmes.

INTRODUCTION

Sugarcane is an important cash crop of Pakistan. Its yield is very low as compared to other sugar producing countries of the world. Among the various factors responsible for low yield, sugarcane borers, *Sciropophaga nivella*, *Chilo infuscatellus* and *Emmalocera depressella* play an important role in restricting the production of sugarcane in Pakistan (Ashraf & Fatima, 1980,1983). The control of sugarcane borers by insecticides has always been difficult due to the concealed feeding habit of the damaging larvae. Moreover, the insecticides cause toxicity and environmental pollution problems. Biological control through parasitoids is the best alternative and a novel method. This method is non-polluting, species specific and its effects are cumulative and long lasting in reducing the pest population. *Trichogramma*, a polyphagous egg parasite, has been successfully used for control of many Lepidopterous pests including sugarcane borers (Ingram *et al.*,1951; Cock, 1985; Cheng, 1986; Hamid &Mohyuddin, 1989; Ashraf *et al.*, 1993). This paper describes the effectiveness of *T. chilonis* for area-wide control of sugarcane borers.

MATERIALS AND METHODS

The parasitoid, *T. chilonis* was mass - cultured on the eggs of Angoumois grain moth, *Sitotroga cerealella* in the laboratory at 25 ± 1^0 C and 60-70% relative humidity. The eggs of the host were collected by confining adult moths in plastic jars (13 x 11 cm) with 20 mesh wire gauze at the bottom and placed on starch in the petri dishes. The eggs were sieved and glued to green, yellow or white paper cards measuring 6x4 cm (Ashraf *et al.*, 1992). These cards were exposed to parasitoids in glass jars for 24 hours. A male and female ratio of 1:5 was maintained for mass-rearing of parasitoids so as to obtain maximum number of females for release programme (Fatima *et al.*, 1992).

An area of 7,100, 27,000 and 30,000 acres of sugarcane during 1992-93, 1993-94 and 1994-95 seasons respectively was earmarked for treatment with parasitoids in Al-Noor Sugar Mills zone at Moro. BL-4 and L-116 were the major varieties of sugarcane grown in this area. The crop was maintained at normal agricultural inputs and no control measure was adopted for sugarcane borers. The parasitoids were released in the field by attaching the cards to the leaves of sugarcane prior to adult emergence @ 10,000 per acre. The releases of parasitoids in sugarcane fields were inundated during the growth period of the crop from April to October. Separate control check plots were also maintained where no parasites were released.

The egg clusters of sugarcane borers were collected regularly to record the post-release performance of parasitoids in the field. In the early growth period of the sugarcane crop from March to June, the borer infestation was determined on the basis of dead-hearts, by examining 200-300 shoots at random in each field. At the harvest of the crop from October to March, the assessment of borer damage was made on internodes affected both in treated and check fields. Twenty cane from each corner and centre of the field were harvested at random and evaluated for borer damage.

RESULTS AND DISCUSSION

The observations on post-release performance of *T. chilonis* indicated that the borer eggs were effectively parasitized, resulting in the decline of the borer population in the field. The mean parasitism recovery was 42.9% in the month of April, decreasing to 36.9% during May. The parasitism recovery was almost constant during June and July (41.8 and 41.7% respectively) which thereafter increased to 67.9% in August, 1993 (Table 1). Hamid & Mohyuddin (1989) reported almost 100% parasitism in the eggs of *C. infuscatellus* in the month of August after the releases of *T. chilonis*, which resulted in a significant reduction in borer damage.

Table 1. Percent recovery of *Trichogramma chilonis* from sugarcane borer eggs collected from different treated areas.

Month (1993)	Mean parasitism recovery (%)
April	42.9
May	36.9
June	41.8
July	41.7
August	67.9
September	64.6

The results obtained for three years from 1992-95 on the effectiveness of inundative releases of parasitoids revealed that borer infestation in the areas where *Trichogramma* were released remained quite low in comparison with control sites. This indicated that *T. chilonis* effectively checked the build up of borer populations in the treated fields. The borer infestation on the growing crop from March to June (Table 2) in the treated areas ranged from 5-12% dead-heart incidence throughout the observation period as against 15 - 40% in untreated control. The mean borer damage (on the basis of internodes affected) at the harvest of the crop (1992-95) in treated areas remained under the economic threshold level (10%) and ranged from 3.2 - 4.1%. In the check field where no *Trichogramma* were released the average borer damage ranged from 9.1 - 32.7% during the period of study (Table 3). The reduction in borer damage due to the inundative

releases of parasitoids are supported well by the findings of many research workers (Cheng, 1986; Hamid &Mohyuddin, 1989; Ashraf *et al.*, 1993) who also recorded significant reduction of borer damage in plots where *T. chilonis* were released.

Table 2. Borer infestation on the basis of dead-hearts in sugarcane treated with *Trichogramma chilonis.*

Month (1993)	Borer infestation (% incidence)	
	Range	Mean
March	5-10	7.7
April	7-10	8.0
May	7-12	9.8
June	8-10	9.1
Control	15-40	27.0

Table 3. Area-wide control of sugarcane borers in Al-Noor Sugar Mills by inundative releases of *Trichogramma chilonis.*

Seasons	Area treated (acres)	Mean borer infestation on internode basis (%)	
		Treated	Control
1992-93	7,100	4.1	9.1
1993-94	27,000	3.2	32.7
1994-95	30,000	3.3	29.9

The parasite recovery from the egg masses of sugarcane borers indicated the establishment of parasitoids in the target area. These studies manifest the effectiveness of this parasitoid in controlling sugarcane borers on one hand and reducing pesticide application on the other. Furthermore, *T. chilonis* has great potential for its inclusion in the integrated management of sugarcane borers for area-wide control programmes.

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DEPLOYMENT OF *PASTEURIA PENETRANS* FOR THE CONTROL OF ROOT-KNOT NEMATODES IN ECUADOR

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ABSTRACT

An isolate of *Pasteuria penetrans* (Pp) found at the Experimental Station in Boliche, Ecuador, was used to treat field plots. The progressive development of the Pp infection was monitored over 6 successive crop cycles of beans and tomatoes which were susceptible to the naturally occurring root-knot nematode populations (*Meloidogyne incognita*). In the Pp treated plots the incidence of root galling was less than in the untreated, this was also reflected in increased crop growth. At the end of the experiment the numbers of free living infective juveniles in soil had decreased in the treated plots, and those that were recovered from soil samples were encumbered with Pp spores.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are a serious constraint to the production of vegetables in Ecuador. This is because susceptible crops are grown regularly in the same land sometimes in monoculture or in rotations of vegetables, all of which are hosts to root-knot nematodes. Conditions are generally good for vegetable production in all the climates and ecological zones of the country, allowing root-knot nematodes to thrive also. Commercial producers often use nematicides, but their frequent use is being questioned because of declining efficacy and possible undesirable environmental effects. Because of the limited range of crops grown (none of which are non-hosts of *Meloidogyne* spp.) the use of rotations as a control strategy is limited. Similarly, nematode-resistant varieties are few and not always appropriate for the conditions or the market. Interest is therefore developing in methods of biological control and prospects for developing suppressiveness to the nematodes.

Pasteuria penetrans, an obligate, bacterial parasite of root-knot nematodes, has been recorded from many countries and is considered to be one of the more promising biological control agents (Mankau, 1975, Stirling, 1984). Its natural occurrence in soil, although widespread, is often at low levels and detection can be difficult; its presence is probably frequently overlooked. Furthermore, the concentration of *P. penetrans* spores may be influenced by variability in the susceptibility of host nematodes to particular populations (Gowen & Tzortzakakis, 1994).

Suppressiveness to root-knot nematodes can sometimes be induced in soils by repeated planting of susceptible crops, particularly when a biocontrol agent has been applied or is known to be present (Barker & Cook, 1974, Channer & Gowen, 1992).

The objective of this study was to see if an initial application of *Pasteuria* spores to top soil in plots infested with root-knot nematodes would increase over a series of crop cycles and decrease the population density of the nematodes.

MATERIALS AND METHODS

The experiment was carried out at the Boliche Experimental Station of the Instituto Nacional de Investigaciones Agropecuarius (INIAP) in the coastal region of Ecuador 26 km east of Guayaquil. The experimental site is 17 m above sea level with a silty clay loam soil, pH 6.2 and 1.15% organic matter. The climate in this region is characterised by a 4-month rainy season, January-April, with an average of 1700 mm and a long dry season, May-December. Mean minimum and maximum temperatures are 20 and 33°C respectively and r.h. is 64-97%. The site was chosen because it was heavily infested with root-knot nematodes (*M. incognita and M. javanica*). *P. penetrans* was detected on free living J_2 juveniles but at a low incidence. The experiment was done in 1 x 1 m microplots prepared at the site. Plots were contained with wooden barriers and they were separated by 1 m paths. Treatments were designed to promote the rapid increase of Pp through the continuous cultivation of root-knot nematode susceptible crops at normal and above-normal planting densities and with two levels of *P. penetrans* spores applied to the upper 15 cm of soil at the start of the experiment.

The treatments were as follows:

1. Normal density cropping (four plots/m^2)
2. High density cropping (four plots/m^2)
3. Normal density cropping plus 4 x 10^7 spores of *P. penetrans*
4. High density cropping plus 8 x 10^7 spores of *P. penetrans*

Treatments were in a randomised block design with six replicates. The spore inoculum of *P. penetrans* was derived from a population that had been found at Boliche. Spores were mass-produced on tomato plants following the method described by Stirling & Wachtel (1980). The spore preparation contained in powdered tomato roots was mixed with soil in the microplots prior to sowing plots with the first crop of *Phaseolus vulgaris* INIAP 472.
The sequence of cropping began in August 1993 and continued until December 1995: the sequence was as follows:- *Phaseolus, Phaseolus,* tomato, *Phaseolus, Phaseolus,* tomato. The tomato variety used was Walter, an indeterminate variety widely grown in Ecuador.
At the end of each crop, measurements were taken of plant growth and yield and the levels of nematode infestation and presence of *P. penetrans* were recorded.

Soil samples were taken from each plot and nematodes were extracted from 100 cm^3 aliquots using modified Baermann extraction dishes. Spore attachment was recorded on 50 juveniles from each plot. Root systems were removed and assessed for galling using a 0-10 scale (Bridge & Page 1980). They were then dried and milled and the concentration of *P. penetrans* spores estimated in the powdered root samples. After each crop, the root systems were returned to their respective plots.

RESULTS

Data collected at the end of the sixth crop cycle (tomato) are presented in Tables 1 & 2. In those treatments in which Pp was applied, the intensity of root galling was significantly less than those from untreated plots. Similarly, there were lower numbers of free-living juveniles in the treated plots. The differences in the treatments were also reflected in the fruit yield,

which was significantly higher in those plots treated with *P. penetrans* . The effects of planting density and size of initial Pp inoculum were not significant except that where *P. penetrans* was not applied, yields were higher in the normal plant density plots than with the high plant density (Table 1).

Table 1. Root-knot nematode galling indices, numbers of free-living juveniles and yields of tomato in the sixth crop cycle after treatment with spores of *Pasteuria penetrans*.

Treatment	Gall index (0-10)	Juveniles/ 100 cm^3 soil	Yield kg/plot
Normal plant density	8.1	1267	4.8
High plant density	9.0	1083	4.1
Normal density + 4 x 10^7 spores	4.8	317	5.8
High density + 8 x 10^7 spores	5.3	258	5.9
SED	0.47	267	0.07

In the Pp-treated plots, almost all of the nematodes extracted from the soil sample were encumbered with 1 or more spores (Table 2). Between 25 and 32% of these had >10 spores, an infection intensity not found in the untreated plots. In the previously untreated plots, 25-30% of the juveniles had 1-10 spores indicating that there was a natural *P. penetrans* population at the experimental site. This had increased progressively over the course of the experiment (data not presented). The concentrations of *P. penetrans* spores in the dried roots were significantly greater in the treated plots, with a 10-fold difference relative to those in roots from untreated plots.

Table 2. Levels of spore attachment on 50 *Meloidogyne incognita* juveniles extracted from microplots and spore concentrations in root systems at completion of sixth crop cycle.

Treatment	Spores/juvenile			Spores/mg dry tomato root (x 10^4)
	0	1-10	>10	
Normal plant density	41	9	0	4.8
High plant density	37	13	0	4.5
Normal density + 4 x 10^7 spores	1	37	12	95.0
High density + 8 x 10^7 spores	0	42	8	52.0

DISCUSSION

Over 28 months of cropping with root-knot nematode-susceptible varieties of bean and tomato in heavily infested land, treatments with spores of *P. penetrans* effectively suppressed the nematodes, leading to an improvement in crop yield of at least 20%. The reasons why this treatment was so successful at this location are as yet unresolved. Similar effects are known from Florida where the experimental site is on a sandy soil (Oostendorp *et al.*, 1991, Weibelzahl-Fulton *et al.*, 1996) and in Crete where an experiment was conducted in a

commercial plastic-covered tunnel (Tzortzakakis & Gowen, 1994). Some results elsewhere have been less conclusive (Daudi *et al.*, 1990).

The attempt to induce suppressiveness by doubling the plant density was not effective in increasing the concentration of *P. penetrans*, neither was the double dosage of spores applied at the beginning of the experiment.

It may be significant that the population of *P. penetrans* used was endemic to the Experimental Station; work in other countries has been done with introduced populations (Daudi *et al*, 1990). Similarly, the method of distributing the initial spore inoculum and of returning root systems to the plots at the end of each crop cycle may have been beneficial. These issues need further evaluation. *P. penetrans* was present in the untreated plots at the site, and it would have been useful if the experiment could have been continued to monitor the *P. penetrans* in the control plots.

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THE DEVELOPMENT OF *PASTEURIA PENETRANS* AS AFFECTED BY DIFFERENT PLANT HOSTS

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ABSTRACT

The obligate endospore-forming parasite *Pasteuria penetrans* has been successfully used for control of root-knot nematodes. The development of the nematodes is affected by the plant-host.Three experiments were conducted to determine whether the plant-host could also affect the development of the parasite or whether the number of endospores produced within nematode (*Meloidogyne javanica*) females differed between plant - hosts. Tomato, tobacco and banana were compared in the first experiment, tomato, egg plant and okra in the second and tomato and tobacco in the third. The development of the parasite as well as the number of endospores that developed in individual females was found to differ significantly between some of the hosts, with the highest number of endospores being produced in females reared in okra roots.

INTRODUCTION

Root-knot nematodes have shown differential ability to invade and reproduce on different plant-hosts or even cultivars of the same host (Hadisoeganda & Sasser, 1982). This feature is closely related to the resistance that some hosts or cultivars have expressed against root-knot nematodes. Since the plant-host influences the dynamics of reproduction of root-knot nematodes, it could also influence the dynamics of parasitism by *Pasteuria penetrans*. Failure of the parasite to establish in its host or to reach the final endospore-forming stage has been noted even where initial vegetative stages have been observed (Wilkins, 1995).
This study was conducted to determine the effect of the plant-host on the development of parasitized females of root-knot nematodes. A second aim of the study was to monitor the amount of endospores produced in females reared in each plant-host.

MATERIALS AND METHODS

In two experiments an isolate of *P. penetrans* (designated as Pp3) originating from South Africa was used and a population of *Meloidogyne javanica* originating from Malawi on tomato cv. "Tiny Tim". The *Pasteuria* spore suspension was prepared using the method described by Stirling & Wachtel (1980). Eggs of *M. javanica* were removed from a tomato root sytem using the hypochlorite method described by Hussey & Barker (1973) and newly- hatched juveniles were collected from modified Baermann trays (Southey, 1986). All plants were placed, after inoculation, in a growth room, with 16h light and 8h dark and temperatures of 30^0C and 23^0C

respectively. Plants were harvested after a certain amount of degree-days had accumulated (base temperature was 10^0C) (Stirling ,1981).

Experiment 1

Ten ml of a spore suspension of *P.penetrans* containing $1.6x10^5$ spores/ml was added to 60ml of a suspension of *M. javanica* containing 60,000 J2s. The suspension was incubated at 28^0C and spore attachment was monitored until 90% of juveniles were encumbered with 5-8 spores. The suspension was then poured through a 20µm sieve to separate nematodes from spores and the juveniles were washed from the sieves and collected in 300ml of water. Tomato plants (cv. "Tiny Tim"), oriental tobacco (cv. "Sampsun") and Cavendish bananas (cv. "Grand Nain"), were grown in loam-based compost (John Innes No2) in 1 litre plastic pots. Five ml of suspension containing 900 encumbered J2s were used for inoculating 16 plants of each plant-host. Four plants of each host were harvested over 4 different dates.

Experiment 2

Ten ml of a spore suspension of *P. penetrans* containing $1.6x10^5$ spores/ml was added to 50ml of a suspension of *M.javanica* containing 55,000 J2s. The suspension was incubated at 28^0C and spore attachment monitored until 90% of juveniles were encumbered with 6-9 spores, at which point the suspension was poured through a 20µm sieve. The juveniles were washed from the sieve and collected in 350ml of water. Tomato plants (cv. "Tiny Tim"), okra (cv. "Ladies Fingers") and egg plant (cv. "Money Maker"), were grown in loam based compost in 1l plastic pots. Five ml of the above suspension containing 750 encumbered J2s were used for inoculating 20 plants of each plant-host. Five plants of each host were harvested over four different dates.

Experiment 3

The same procedure, as in the previous experiments, was followed in which five ml of a suspension containing 300 J2s encumbered with 6-10 spores per juvenile were used for inoculating five tomato and tobacco plants.

ASSESSMENT OF THE EXPERIMENTS

After the pre-selected numbers of degree-days (650-800) had been accumulated, plants were harvested and the roots were washed free of soil. An estimation of the developmental stage was obtained by picking 10 and 20 selected females per root system for the first and second experiment respectively. Each female was placed in a drop of water on a microscope slide and squashed with a cover slip. These were immediately examined under a light microscope at 400x magnification. The presence of endospores and vegetative stages (Sayre & Wergin, 1977) was used to monitor the development of *P.penetrans* based on the key presented in Table 1. From the plants harvested after 800 degree-days, five groups of 10 and 20 selected females were collected from each plant for the first and second experiment respectively. Each group was placed into a 2.5cm Petri dish in 1ml water. Their cuticles were ruptured using forceps and scalpel under a dissecting microscope and the body contents were dispersed. This suspension was placed in a 50ml conical flask and 9ml of water was used to rinse off the Petri dish into

the flask. The ten ml suspensions were placed on a shaker for half an hour. After this period, six samples of each suspension were taken for counting mature endospores using a haemocytometer. The mean numbers were expressed as thousands of endospores per female. The plants of the third experiment were harvested after 800 degree-days had accumulated. Ten selected females were collected from each plant and each female was placed into a 2.5cm Petri dish in 1ml water. The process was the same as before but the final 10ml suspension was contained spores coming from only one female.

Table1. Keys for estimating the development of *Pasteuria penetrans* inside females of root-knot nematodes

0: no infection
1: microcolonies the predominant stage
2: microcolonies plus quartets
3: quartets the predominant stage
4: quartets plus immature spores
5: immature spores the predominant stage
6: immature plus mature spores
7: mature spores the predominant stage

RESULTS

Experiment 1

There was no significant difference in the rate of development of *P.penetrans* between the three hosts at each sampling occasion (Table 2). Although at the first sampling (after 650 degree-days), there were apparent differences in the development of the parasite inside banana roots compared with the other two plant-hosts, this was not statistically significant.

Table 2. The development of *Pasteuria penetrans* inside *Meloidogyne javanica* reared on tomato, tobacco and banana, according to the amount of degree-days accumulated

Plant	Degree-days from inoculation			
host	650	700	750	800
tomato	5.71	6.04	6.82	6.87
tobacco	5.81	6.28	6.84	6.86
banana	6.60	6.56	6.80	6.95
SED	0.378	0.329	0.099	0.112
n=4				
P	>0.05	>0.05	>0.05	>0.05

The number of mature endospores produced in *M.javanica* females inside the three hosts did not differ over the three host-plants (Table 3). The plant-host in which females were reared thus did not influence the production of *Pasteuria penetrans*.

Table 3. Numbers of mature endospores (in thousands per female) produced inside *Meloidogyne javanica* reared on tomato, tobacco and banana, after 800 degree-days

HOST		
tomato	tobacco	banana
433	487	565
(2.59)	(2.61)	(2.73)

SED = (0.171), n=4
numbers within brackets represent $\log_{10}x$ transformations

Experiment 2

The development of *P. penetrans* has shown statistically significant (P=<0.05) differences between plant-hosts (Table 4), but only in the first sampling date after 575 degree-days. The rate of development was faster in females reared on egg plant than on tomato and okra.

Table 4. The development of *Pasteuria penetrans* inside *Meloidogyne javanica* reared on tomato, okra and egg plant according to the amount of accumulated degree-days

Plant	degree-days from inoculation			
host	575	650	725	800
tomato	3.66	5.56	6.20	6.72
okra	4.03	5.99	6.27	6.83
egg plant	4.55	5.84	6.28	6.77
SED	0.203	0.269	0.098	0.058
n=5				
P	<0.05	>0.05	>0.05	>0.05

The number of mature endospores produced differed significantly (P=<0.05) between the three plant-hosts (Table 5). The highest number of endospores was produced in females reared in okra roots, while the lowest numbers were produced in egg plant roots.

Table 5. Numbers of mature endospores (in thousands per female) produced inside *Meloidogyne javanica* reared on tomato, okra and egg plant after 800 degree-days

HOST		
tomato	okra	egg plant
507	678	398
(2.702)	(2.830)	(2.591)

SED = (0.0484), n=5
numbers within brackets represent $\log_{10}x$ transformations

Experiment 3

The number of mature endospores produced in *M. javanica* females inside the two hosts did not differ on the two host-plants (Table 6), results which agree with the results from the first experiment.

Table 6. Numbers of mature endospores (in thousands per female) produced inside *Meloidogyne javanica* reared on tomato and tobacco after 800 degree-days

HOST	
tomato	tobacco
579	569
(5.755)	(5.747)

SED=(0.0689), n=5
numbers within brackets represent $\log_{10}x$ transformations

DISCUSSION

The life cycle of *P. penetrans* can be completed on *M. javanica* infecting all the plant-hosts examined in this study. Although initially the development of the parasite was faster in *M. javanica* infecting egg plant, there was no subsequent difference between hosts. It was clear that under the specific experimental conditions, at least 750 degree-days are needed in order for most of the parasitized females to reach the "mature endospores" stage (Tables 2,4). This is crucial when an *in vivo* technique is used for multiplication of the spore inoculum, although early harvesting is preferred for infectivity tests in order to avoid confusion with subsequent generations of root-knot nematodes.

Okra would appear to be the most appropriate of the plant-hosts examined for mass production of endospores of *Pasteuria penetrans*. It was observed that more females developed in tomato root systems than those of the other plant-hosts. So the higher number of endospores produced in okra roots could be due to greater food availability in the individual host nematode though decreased competition for food since fewer females have been established in the root of this plant. On the other hand, the invasion rate was approximately the same between tomato and egg plant roots but this time the numbers of endospores produced in the first host were significantly higher than the second one, suggesting that other host factors may play a role in determining reproduction of *P. penetrans*.

A question which has been raised from this study is what is the optimum level of invaded parasitized females required in order to obtain maximum numbers of endospores using different plant-hosts? More research is needed towards this direction, and monitoring the development of the parasite at earlier sampling dates should be considered.

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BIOLOGICAL CHARACTERISATION OF *RHIZOCTONIA SOLANI* IN RICE-BASED CROPPING SYSTEMS

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ABSTRACT

The diversity of populations of pathogenic *R. solani* in rice-based systems in south-east Asia and west Africa was investigated by means of anastomosis grouping, morphological, biochemical and molecular diagnostic techniques. Multi-disciplinary characterisation of 31 *Rhizoctonia solani* isolates from rice-based cropping systems showed that the majority of isolates belonged to anastomosis group 1, but morphologically was highly variable, with no consistent characters that related to host of origin, production system or geographic region. Pathogenicity testing on rice, soybean and a range of weed species indicated that these strains showed little host specificity. Isolate sub-groupings were identified by analyses of genetic similarity through PCR-RAPDs and mitochondrial DNA RFLPs, and by analysis of pectic enzyme production. Some of the groupings identified showed good cross-correlation between the different approaches, supporting the hypothesis that anastomosis group 1 is not a homogenous group. The genetic differences underlying these populations provide a useful means of examining the nature and spread of populations within rice systems.

INTRODUCTION

Rhizoctonia solani (sensu lato) is a ubiquitous and diverse plant pathogen, causing extensive losses to most annual and many perennial crops, particularly at the seedling stage. Primarily soil-borne, *R. solani* also incites diseases of aerial plant and is of particular importance to subsistence farmers where a range of crops may be attacked within a mixed farming system. *R.solani* is considered a collective species, consisting of a number of loosely-related strains which have been grouped into 11 anastomosis groups (AGs). Further intraspecific groups (ISGs) have been established in several AGs based on morphology, pathology and other criteria. However, the taxonomic status of AGs and ISGs has not yet been resolved, and these groups may represent separate species (Vilgalys & Cubeta, 1994). While considerable research has been undertaken to study the diversity of *R. solani* at the AG level, the degree of variation present within ISGs at population level and among individual strains is still unknown, although this knowledge is critical to the development of disease management strategies. The lack of suitable methods for identifying groups of isolates and individual strains has been a major obstacle. Rice sheath blight, caused by *R. solani*, has become economically-important disease in intensive rice production in much of Asia and west Africa due to the increased use of fertilisers and of new varieties with greater tillering capacity (IRRI, 1995). Traditionally, the sheath blight pathogen has been associated with isolates of AG-1 1A. Although considerable research has been undertaken in temperate countries to study the disease development and epidemiology of this pathogen, only a few recent

investigations have concentrated on the diversity of isolates of *R. solani* from tropical rice-based cropping systems (e.g. Liu & Sinclair, 1993). Understanding of the diversity of the pathogen in these ecosystems is urgently required to enable breeding of varieties with appropriate resistance and the development of biological control strategies utilising antagonistic micro-organisms. An extensive research programme has been undertaken at the International Mycological Institute to further develop biochemical and molecular genetic techniques to resolve this. The programme involved a detailed study of the diversity of *R. solani* in rice-based cropping systems in SE Asia and W Africa and results of this multi-disciplinary approach to characterisation of the pathogen are presented here.

MATERIALS AND METHODS

A comprehensive collection of tropical and subtropical isolates of *R.solani* occurring in rice-based cropping systems was established at the International Mycological Institute. A representative set of 31 strains was selected for the study (Table 1) and maintained on PDA at 25°C. Anastomosis grouping was conducted with tester strains of AG-1, AG-2 1, AG-2 2 and AG-4 using an adaptation of the clean slide technique described by Kronland and Stanghellini (1988). Thirty morphological characters were categorised, including culture pigmentation and various characteristics of hyphae and sclerotia. Isolates were subcultured onto PDA, incubated at 25°C and the morphology of each isolate evaluated after four weeks. Data were analysed by unweighted pair group analysis (UPGMA) using Gower's coefficient (MVSP, Plus-Version 2.0, Kovach, 1989-1990). Pectic enzymes, produced by isolates after incubation at 25°C in a pectin broth, were detected by electrophoresis in a pectin polyacrylamide gel following the method described by Cruickshank (1990). Isoenzymes were visualised by staining with ruthenium red, overall enzymatic patterns assessed for each isolate and isolates grouped accordingly. Total DNA was extracted from isolates of *R. solani* (procedures adapted from methods described by Raeder & Broda, 1985, and Vilgalys and Hester, 1990) and digested with the restriction enzyme *Hae*III (Gibco BRL). Mt DNA fragments were separated by gel electrophoresis in 1% (w/v) agarose gel at a constant voltage of 5 V cm-1. Total DNA of a subsample of 23 isolates was amplified by PCR using the primers MR, RY and GF (Bridge *et al.*, 1996). Primers were used at 0.134 µmol l^{-1} with 1.5 mmol l^{-1} $MgCl_2$ and $5x10^{-3}$ U μl^{-1} Tth enzyme (HT Biotechnology) in 10x Tth buffer. PCR amplification was performed under a program consisting of 45 cycles, with stages of 60 s at 95°C, 60 s at 35°C and 60 s at 72°C, followed by a final single cycle of five minutes at 72°C. Amplification products were separated by electrophoresis in a 1.5 % (w/v) agarose gel at a constant voltage of 5 V cm-1. Gels from both types of DNA analysis were stained in ethidium bromide (0.5 µg ml^{-1}) and visualised under UV light. Characteristic fragments obtained from both approaches were identified and the presence or absence of each band recorded for each isolate. The data matrices were analysed separately by unweighted pair group analysis (UPGMA) using Gower's coefficient. Pathogenicity of isolates of *R. solani* was determined under glasshouse conditions on two varieties of *Oryza sativa* and *Glycine max.* and the weed species *Eleusine indica*. Eight replicate plants were inoculated with each isolate, using colonised millet grain inoculum. Severity was recored on these hosts by measurement of the extent of colonisation. Disease incidence on a further seven weed species, inoculated with a subset of isolates, was also determined.

RESULTS

Anastomosis groups were successfully determined for 26 isolates, all of which fused with the tester strain of AG-1 (Table 1). The remaining five isolates (IMI 360016, 360298, 360336, 361174, 369673) failed to anastomose with any AG tester strains. Pigmentation of culture colonies was highly variable (ranging from cream to dark brown), as were the number, size and distribution of sclerotia. However, the morphology of most isolates corresponded to those AG-1 1A characters described by Sherwood (1969).

Table 1. Grouping of *R. solani* isolates based on anastomosis group (AG) testing, analyses of RFLPs and pectin isoenzyme patterns.

IMI accession number	Host	Geographic origin	AG	RFLP pattern		Pectin zymogram pattern	
				Group	Subgroup	Group	Subgroup
360337	Grass	Vietnam	1	1	1	1	1
360302	Grass	Vietnam	1	1	1	1	1
358755	*Oryza sativa*	Philippines	1	1	1	1	1
360045	*Oryza sativa*	Vietnam	1	1	1	1	3
303152	*Oryza sativa*	Japan	1-1A	1	1	1	3
360046	Unknown	Japan	1	1	1	1	3
361174	*Oryza sativa*	Nigeria	NI	1	1	1	6
360305	*Oryza sativa*	Vietnam	1	1	1	1	8
361179	*Oryza sativa*	Nigeria	1	1	2	1	4
360366	*Oryza sativa*	Vietnam	1	1	3	1	3
360378	*Oryza sativa*	Nigeria	1	1	3	1	4
360321	*Zea mays*	Vietnam	1	1	3	1	6
369671	*Oryza sativa*	Nigeria	1	1	4	1	10
361178	*Oryza sativa*	Nigeria	1	1	-	1	10
361189	*Oryza sativa*	Malaysia	1	1	5	1	2
361190	*Oryza sativa*	Malaysia	1	1	5	1	2
359620	*Oryza sativa*	Vietnam	1	2	-	1	3
360023	*Oryza sativa*	Cote d'Ivoire	1	2	-	1	3
360021	*Commelina*	Cote d'Ivoire	1	2	-	1	6
360037	*Oryza sativa*	Cote d'Ivoire	1	3	-	1	3
360038	*Oryza sativa*	Cote d'Ivoire	1	3	-	1	8
369673	Soil	Benin	NI	4	-	1	9
360314	*Oryza sativa*	Ghana	1	5	-	2	-
360016	*Vigna ung.*	Benin	NI	6	-	1	5
360377	*Lycopersicon*	Vietnam	1	7	-	2	-
360336	*Brassica oler.*	Vietnam	NI	8	-	3	-
360298	*Brassica chin.*	Vietnam	NI	8	-	3	-
361171	*Oryza sativa*	Nigeria	1	-	-	1	4
358753	*Vigna radiata*	Philippines	1	-	-	1	7
361181	*Oryza sativa*	Nigeria	1	-	-	1	9
359622	*Vigna ung.*	Benin	1	-	-	1	10

NI = Anastomosis group not identified

Cluster analysis of morphological data summarised as a dendrogram (Figure 1) showed that levels of similarity between isolates ranged from 33 to 93 %. No two isolates were identical. One major group was delimited, comprising 19 isolates with similarities above 52 %. Two small isolate clusters were linked to this group (47 and 40 % similarity respectively). A distinct group comprising two isolates (IMI 358753, 359622), showing only 33 % similarity

Fig.1. Dendrogram obtained from Gower's coefficient after UPGMA clustering of data for 30 morphological characters exhibited by *Rhizoctonia solani*

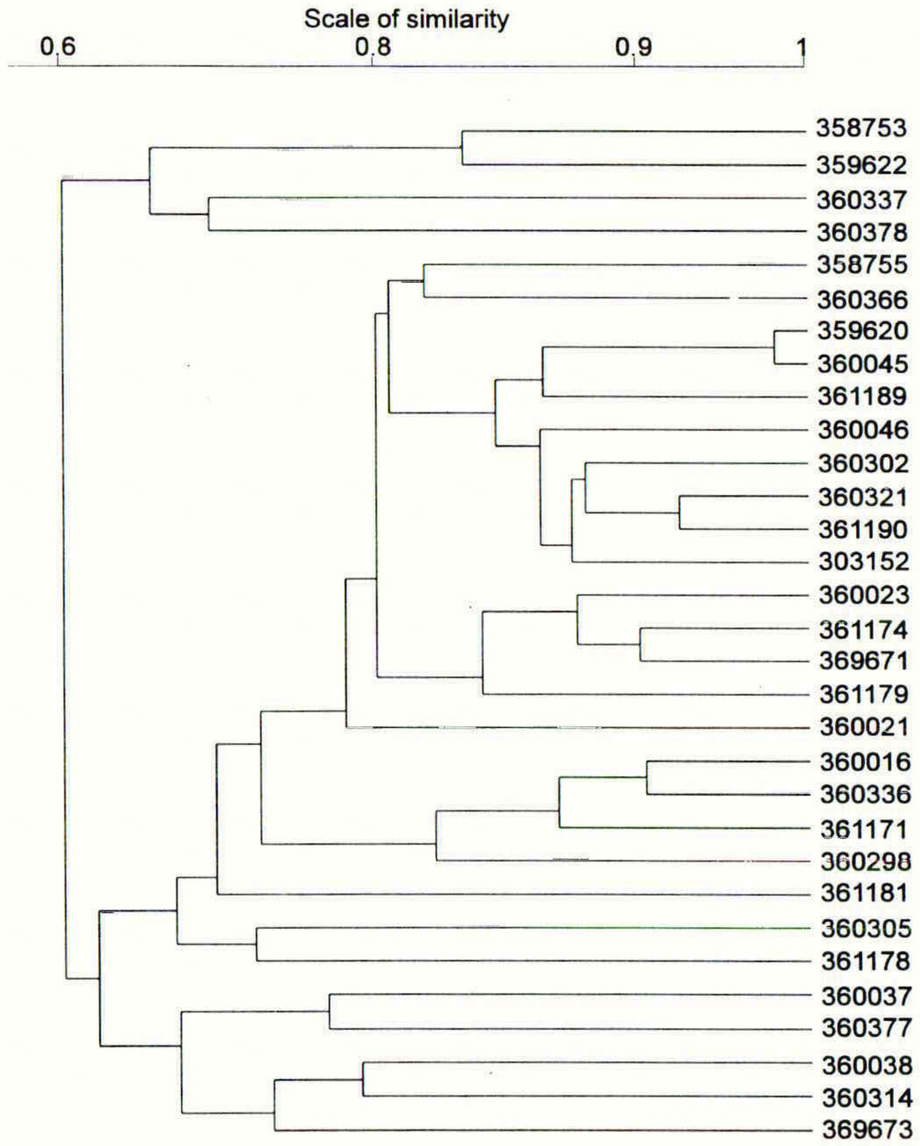
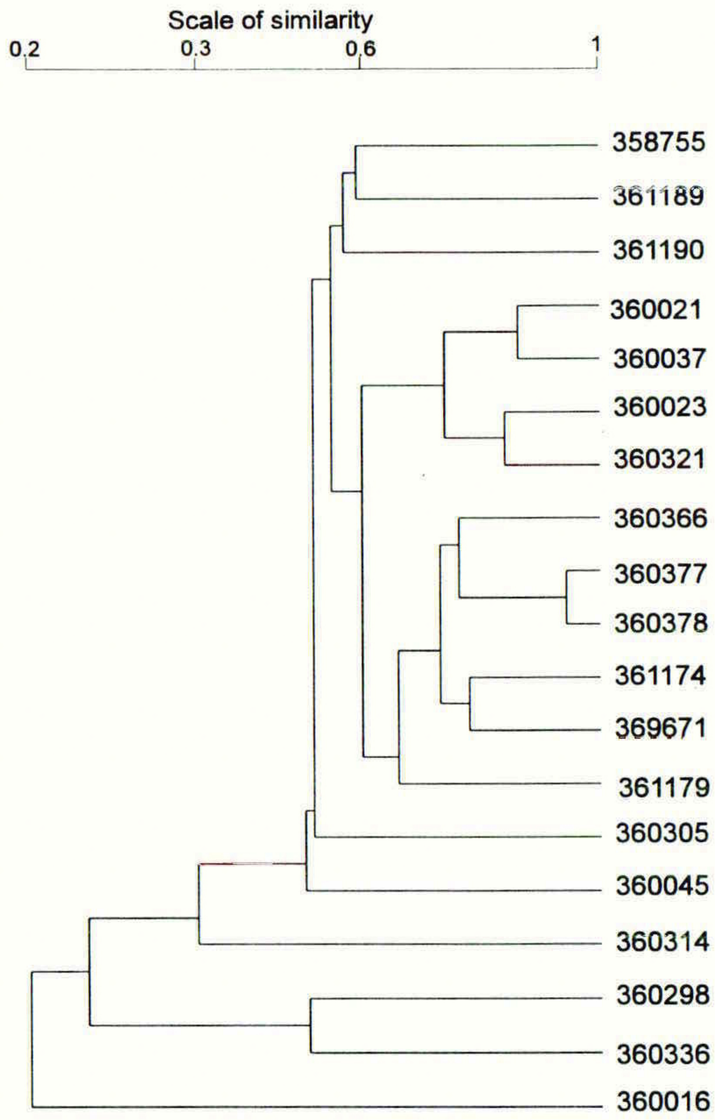


Fig.2. Dendrogram obtained from Percent similarity coefficient after UPGMA clustering of band data generated by PCR amplification of *Rhizoctonia solani* DNA by primers MR, RY and GF



to all other isolates, was also identified. Analysis of pectic enzyme patterns also delimited one major group, comprising 27 isolates, which produced characteristic zymograms (Table 1). Within this group, slight variations in banding patterns indicated the existence of isolate subgroups. Two zymogram groups each comprising two isolates (IMI 360377 and 360314; 360336 and 360298) produced zymograms which were very distinct from the main group. RFLPs were successfully obtained for 27 isolates, with a large group of 16 isolates being identified (52 - 100 % similarity) (Table 1). The banding patterns in this group varied around five characteristic bands, suggesting further subgroups. A second group of isolates (IMI 359620, 360023 and 360021) exhibited three of the five bands obtained from isolates in the above group but also three different fragments. Two isolate pairs (IMI 360037 and 360038; 360298 and 360336), with distinct banding patterns and of very limited similarity to the above groups, could also be identified. Four remaining isolates (IMI 369673, 360314, 360016, 360377) were very distinct from all other isolates and from eachother. The simple repeated motif PCR primers produced a total of 93 distinct DNA electrophoresis bands. Clustering of the data revealed that the majority of isolates formed a single large group, with a number of ungrouped and more distinct isolates primarily from non-rice hosts (Figure 2). The isolate groupings found for each of the primers did not differ to any great extent. Pathogenicity testing revealed that the majority of isolates could infect rice, soybean and the various weed species, resulting in symptoms typical of sheath blight. Two isolates (IMI 360038 and 369673) were non-pathogenic on rice and caused only minor lesions on *E. indica*. Isolate 360366 consistently caused severe symptoms on rice and the monocotyledonous weed species. All isolates caused some degree of damage to soybean. Of the weed species, only *Ageratum conyzoides* remained symptomless. Although disease incidence on the weed species varied depending on the isolate of *R.solani*, all isolates produced symptoms on at least four differing species.

DISCUSSION AND CONCLUSIONS

The majority of isolates, although not all obtained from rice plants, proved to belong to AG-1, thus confirming that the typical pathogen of rice agro-ecosystems is mainly found in this AG. However, strains of AG-1 are not confined to this host plant alone and have been isolated from many other species (Yamaguchi *et al.*, 1984). Nevertheless, none of the isolates which did not anastomose with AG-1 in this experiment, came from rice, indicating that infection of rice by strains from other AGs may be rare in tropical systems. Although most isolates were members of one AG, their morphology was highly variable, indicating that morphologicy alone provides little information on other strain characteristics. Although morphological description for AGs been attempted (Sherwood, 1969), overlapping between groups is inevitable. Some correlation between anastomosis behaviour and pectic zymograms was observed in zymogram group 3. These isolates originated from two different *Brassica* spp from the same location in Vietnam and did not anastomose with any tester strain. However, all other non-AG-1 isolates produced zymograms common to the majority of AG-1 isolates, matching the description by Cruickshank (1990) for AG-1 1A. The variation observed within this zymogram group suggests that AG-1 1A is not a homogeneous group. The isolates in this zymogram group matched those in the major group found by analysis of RFLP and PCR banding patterns. However, variation was again observed around one major pattern, again indicating that AG-1 1A is not homogeneous.

Heterogeneity within AG-1 has also been observed in rDNA internal-transcribed spacer regions. Liu and Sinclair (1993) thus distinguished 6 groups, 4 of which comprised isolates of AG-1 1A. Analysis of RFLPs and PCR banding patterns also confirmed the distinct nature of IMI 360336 and 360298, which were depicted as a separate group in both approaches with a low degree of similarity to other isolates. Pathogenicity testing showed that most of the isolates (including two from *Brassica* spp) were pathogenic on rice and did not differ significantly in virulence. Most isolates were also able to infect a wide range of other plant species, indicating that control of this fungus by crop rotation is likely to be of very limited impact. While a highly virulent isolate (IMI 360366) could not be distinguished from other pathogenic isolates by any of the approaches discussed above, the isolates non pathogenic to rice (IMI 369673 and 360038) showed some distinct characteristics which suggest that diagnostic separation of saprophytes is achievable. Both isolates showed particular morphological features and had distinct RFLP patterns. All approaches used in this study allowed grouping of the isolates into subgroups, indicating that isolates of *R. solani* from rice-based cropping systems do not consist of one homogenous group. However, correlations between groups obtained by the different methods used varied suggesting that no single method is able to give a clear picture of the diversity of *R. solani* in rice.

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SESSION 4D

POSTGRADUATE STUDENT POSTERS

Session Organiser	Dr P E Russell *AgrEvo UK Ltd, Saffron Walden*
Poster Papers	4D-1 to 4D-20

ASSOCIATIONS BETWEEN BLACKPOINT AND GRAIN DEVELOPMENT OF WINTER WHEAT CULTIVARS

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ABSTRACT

Wheat cultivars were monitored from the end of anthesis to maturity, for grain dimensions and moisture content. Blackpoint severity at harvest generally increased with increasing grain dry weight, and was correlated with moisture content and grain width at the late dough growth stage.

INTRODUCTION

Blackpoint describes dark lesions at the embryo end of cereal grain. The causal organism most frequently stated in the UK is *Alternaria alternata* (Culshaw *et al.*, 1988). It has been estimated that 4% of UK wheat grain is downgraded or rejected in an average year due to the disease, and up to 15% in seasons when the disease is more severe (Culshaw *et al.*, 1988; Ellis *et al.*, 1996). Fungicidal control remains inconsistent, so understanding cultivar variation is particularly important. Cultivar differences have sometimes been related to grain size and weight at harvest. This may be because larger grains result in more open florets and allow greater access for fungal spores, or alternatively, larger grains may dry at a slower rate and remain susceptible for longer (Ellis *et al.*, 1996). The investigation outlined here was carried out to study the relationship between grain moisture content and dimensions during maturation and blackpoint severity at harvest.

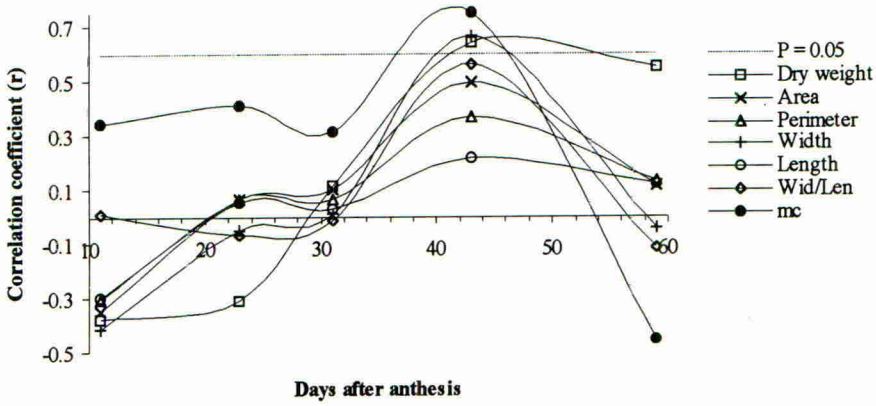
MATERIALS AND METHODS

Mobile plots (60 x 40 x 20cm), filled with a soil nutrient mix (loam:peat:grit) were each sown with one of 11 cvs. of winter wheat at 367 seed/m^2 on 3.10.94. Cultivar plots were randomised within 12 blocks. A harvest of five ears from each plot was made at the average GS 71, 79, 85, 87 and 92 (Zadoks *et al.*, 1974). A single grain was removed from the top, middle and bottom sections of each ear. Grain dimensions were assessed with the use of a computerised image analysis system (Skye Instruments), and the moisture content was calculated after drying at 80°C for 48h. At maturity, grain from each harvested plot were scored for blackpoint (Ellis *et al.*, 1996). Correlation coefficients were calculated between blackpoint and grain dimensions and moisture content during development. Visual checks were made to ensure that significant associations were linear and that data were not grouped.

RESULTS

Correlations between blackpoint and grain dimensions are shown in Fig. 1. With moisture content, the correlation was significantly positive on day 43 (GS 87), but appeared to be

Fig. 1 Correlations between angular transformed total blackpoint score and grain dimensions during maturation (significant at 0.602 or above).



negative, but not significant, on the previous and subsequent assessments. Moisture content on day 43 was also correlated with area (r = 0.632) and width (r = 0.759).

DISCUSSION

This work shows that blackpoint of wheat cultivars can be associated with grain moisture content at specific times during maturation. This is consistent with the hypothesis that blackpoint can be induced by the proliferation of sub-epidermal fungi, such as *Alternaria* spp. under damp conditions (Ellis *et al.*, 1996). The association between moisture content and grain dimensions could explain the previous reports of association between final grain size and blackpoint severity. The lack of direct causal relationship between final grain dimensions and blackpoint might explain the inconsistancy of such relationships (Ellis *et al.*, 1996).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge MAFF for funding this work; Dr V W L Jordan for supervision and Messrs R F Hughes and J B Woodley for technical assistance.

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EFFFICACY OF THE FUNGICIDES PROCHLORAZ AND PYRIMETHANIL AGAINST *FUSARIUM CULMORUM* EAR BLIGHT OF WHEAT

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ABSTRACT

This is the first report demonstrating the use of quantitative polymerase chain reaction (PCR) analysis, in addition to visual disease assessment, to evaluate the efficacy of fungicides against *Fusarium culmorum* ear blight of wheat. According to both techniques, the fungicide prochloraz significantly decreased disease, while the fungicide pyrimethanil had no significant effect. Treatment with prochloraz resulted in a significant increase in 1000 grain weight. A linear relationship existed between 1000 grain weight and disease (visual or quantitative PCR), with yield decreasing as disease increased.

INTRODUCTION

There are suprisingly few reports of successful fungicidal control of *Fusarium* ear blight of wheat. Prochloraz and tebuconazole, two sterol biosynthesis inhibiting fungicides (SBI's), appear to be among the more effective chemicals in glasshouse trials (Hutcheon & Jordan, 1992). New fungicides with different modes of action are continously being developed and may prove useful in controlling FEB of wheat. Pyrimethanil is a new anilinopyrimidine fungicide which is effective against all strains of *Botrytis* (Neumann *et al.*, 1992). The aim of this experiment was to assess the effect of pyrimethanil on *F. culmorum* under glasshouse conditions and to compare its efficacy with that of prochloraz. Also, for the first time, both quantitative PCR and visual disease assessment were used to evaluate the effectiveness of the fungicides.

MATERIALS AND METHODS

Wheat cv. Avalon was grown under glasshouse conditions, 11 plants per treatment. At mid anthesis (GS 65), ears (3 per plant) were inoculated with 7 ml of an *F. culmorum* (strain Fu 42) conidial suspension (10^5 conidia/ml). Prochloraz and pyrimethanil were applied two days post-inoculation (450 g/ha and 400 g/ha, respectively) using a pressurised hand sprayer with a flat fan nozzle, water volumes of 250 l/ha and pressures of 200--300 kPa. Plants were covered with polyethylene bags until harvest to maintain high humidity. Visual disease assessment (% infected spikelets/ear) was performed at GS 80. Ears were harvested at GS 90 and number of grain/ear and 1000 grain weight calculated. The three treated ears from each plant were bulked together, ground to a fine powder, and DNA extracted according to the method of Nicholson *et al.* (1996). Quantitative PCR analysis was performed as described by Lees (1995). Statistical analysis was based on analysis of variance.

RESULTS

According to both visual disease assessment and quantitative PCR analysis results, pyrimethanil had no significant effect on disease (Table 1). However, both techniques suggested that prochloraz-treated samples had significantly less disease than the untreated inoculated controls ($p < 0.001$ & 0.05) (Table 1). Treatment of *F. culmorum* inoculated samples with either prochloraz or pyrimethanil had no significant effect on the mean number of grain/ear (Table 1). However, treatment of *F. culmorum* inoculated samples with prochloraz

resulted in a significant increase in yield, as measured by 1000 grain weight ($p < 0.01$). A linear relationship was observed between the mean 1000 grain weight for each treatment and either the mean amount of *F. culmorum* DNA or the mean disease score ($R^2 = 0.83$ & 0.98, respectively).

Table 1. Effect of prochloraz and pyrimethanil on *Fusarium culmorum* ear blight of wheat.

Treatment	disease score* (%)	*F. culmorum* DNA* (ng/mg plant material)	No. grain/ear*	1000 grain weight* (g)
control	0	0	59.2	31.6
F. culmorum	90.3	0.89	58.1	11.0
prochloraz	0	0	60.7	29.7
pyrimethanil	0	0	57.5	27.8
F. culmorum + prochloraz	64.0	0.51	63.7	15.8
F. culmorum + pyrimethanil	93.6	0.79	62.8	10.6
S.E.M. +/-	4.3	0.1	3.3	2.1

*Mean results obtained for each treatment.

DISCUSSION

Both quantitative PCR and visual disease assessment showed a decrease in *F. culmorum* ear blight of wheat when treated with prochloraz. In *in vitro* fungicide tests, Polley *et al.* (1991) found that prochloraz was particularly effective against the *F. culmorum* isolates tested, and also against *Microdochium nivale* and *F. avenaceum isolates,* two other causal agents of FEB. Pyrimethanil had no significant effect on disease. The effectiveness of prochloraz against *F. culmorum* ear blight was also reflected in the 1000 grain weight results. Treatment of *F. culmorum*-inoculated samples with prochloraz led to a significant increase in 1000 grain weight, while treatment with pyrimethanil had no significant effect. Also, a linear relationship existed between yield (as measured by 1000 grain weight) and disease (as measured by both visual disease assessment and quantitative PCR analysis), with the yield decreasing as the level of disease increased. This experiment has illustrated the potential use of quantitative PCR analysis in evaluating fungicide performance, and experiments are currently being undertaken to further investigate the relationship between visual disease assessment and quantitative PCR analysis.

ACKNOWLEDGEMENTS

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IMPROVED BIOLOGICAL CONTROL OF PEACH TWIG BLIGHT BY PHYSIOLOGICAL MANIPULATION OF *EPICOCCUM NIGRUM*

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ABSTRACT

The performance of *Epicoccum nigrum* as a biocontrol agent of peach twig blight (*Monilinia laxa*) depends on conditions of high relative humidity. Production of *E.nigrum* in medium at reduced water availability (a_w = 0.98) resulted in improved biocontrol, compared with inoculum produced at high water availability (a_w = 0.995). The fungus accumulated compatible solutes when grown at reduced water availability. This accumulation probably contributes to a more efficient use of water when the fungus is placed in the phyllosphere.

INTRODUCTION

Epicoccum nigrum, a component of the resident mycoflora of peach twigs and flowers, reduces twig blight caused by *Monilinia laxa*. However, biocontrol obtained is variable, depending on disease severity and humidity conditions (Madrigal *et al.*, 1994). When exposed to conditions of low water availability, fungal cells accumulate compatible solutes, generally polyols, glycerol being the most important. Recently, it has been shown that conidia of entomopathogenic fungi with elevated concentrations of polyols tolerated lower water potentials and were more pathogenic than unmodified conidia (Hallsworth & Magan, 1994). The present investigation attempted to use this approach to improve biological control by aclimatization of *E.nigrum* to conditions of low water availability in the phyllosphere.

METHOD

Biomass of *E.nigrum* was obtained from 10 d old cultures on potato dextrose agar (PDA) (mainly spores) and potato dextrose broth (PDB) (mainly mycelium). PDA and PDB were unmodified (water activity = 0.995) or modified with glycerol (water activity = 0.98). Polyols were quantified by HPLC, with a Hamilton HC-75 Ca^{2+} column; a refraction index detector; mobile phase of acetonitrile:water (40:60). Mycelium and spores were separated from the culture media, washed and freeze dried. Samples of 50 mg of freeze-dried material were mixed with 1 ml Analar water, sonicated for 2 min, boiled for 5.5 min and filtered through 0.2 μm filters. Samples were then injected in the HPLC. For the field trial, mycelium and spores of *E.nigrum* were separated from the culture media, homogenized in a 0.06 % Nu-Film-17 solution and filtered through cheesecloth. The fungal suspensions were adjusted to 10^6 conidia (plus mycelial fragments)/ml. Treatments were first applied the day before artificial inoculation of peach twigs with *M.laxa*, and applications were then repeated four times at 7-day intervals. A control treatment with captan was set up. Disease was

assessed by measuring the length of lesion induced by *M.laxa* in the shoots.

RESULTS AND DISCUSSION

Disease control by *E.nigrum* was higher when the fungus was produced in media at reduced water activity compared to normal water activity, although neither differed from captan ($p=0.05$) (Figure 1). In these conditions osmotic adjustment probably results in a reduction of cell water potential by accumulation of compatible solutes. Glycerol and arabitol were the solutes accumulated (Table 1), with an ability to depress water activity greater than others such as erythritol or mannitol. When the fungus is then sprayed, the imbalance in water potential with the phyllosphere is reduced compared to the inoculum produced at high water availability. *E.nigrum* becomes less dependent on high atmospheric relative humidity, and an advantageous colonization occurs on the twigs resulting in improved disease control. This demonstrates that quality of inoculants can be modified during the production process by physiological manipulation of antagonists.

Figure 1. Evolution of lesions induced by *M.laxa*.

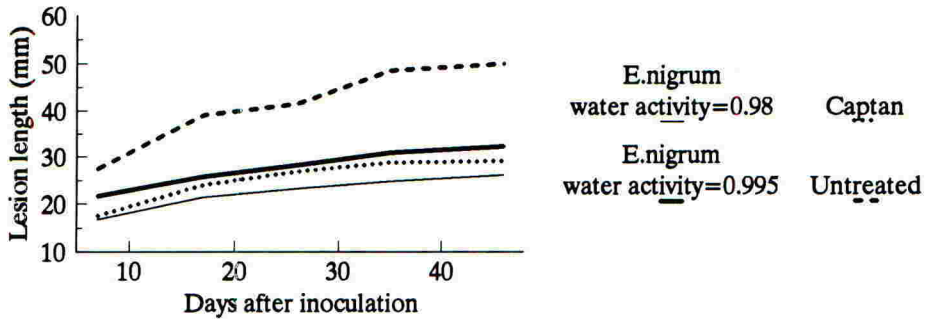

Table 1. Contents of polyols in spores (S) and mycelium (M) of *E.nigrum*. Data are means of three replicates and indicate mg/g freeze-dried biomass. Asterisks indicate a significant difference in the solute analyzed.

water activity of medium (a_w)	glycerol		arabitol		mannitol	
	S	M	S	M	S	M
0.995	15.84	2.38	4.45	22.68	99.93	123.89
0.98	149.31*	382.29*	16.37*	25.41	66.79	82.04

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DRY ROOT ROT OF CITRUS AND ITS RELATIONSHIP TO SOIL PHYSICAL CONDITIONS

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ABSTRACT

Dry root rot of citrus is associated with low soil air permeability and air-filled porosity, and high soil moisture. Poor soil drainage and aeration, and/or high watertables are linked with the incidence of dry root rot.

INTRODUCTION

Dry root rot of citrus (known in Australia as "sudden death"), occurs in all citrus growing areas of New South Wales (NSW), Australia. It affects trees of all scion varieties on *Poncirus trifoliata* and Carrizo and Troyer citrange rootstocks. Trees, often carrying good crops of fruit suddenly wilt and die. Affected trees show several black, rotting structural roots at depth, with a dry brown discolouration that extends into and across the tree butt. This discolouration has an odour typical of rancid coconut. The fungus *Coprinus micaceus* is often found fruiting at the base of collapsed trees. Inoculation of nursery trees with *C. micaceus, Fusarium spp.* and/or *Diaporthe citri* (all isolated from the discoloured wood of affected trees) failed to reproduce the disease (Broadbent & Fraser, 1977) under ideal conditions.

Dry root rot has been associated with poorly aerated and/or poorly drained soil (Fawcett, 1936, Menge, 1989). Field observations in the Murrumbidgee Irrigation Area (MIA) and the Central Coast of NSW support this association. The aim of this research was to examine the relationship of dry root rot with the prevalent soil physical conditions.

MATERIALS AND METHODS

Soil pits located at several MIA orchards were used for the comparison of soil profiles between healthy and dry root rot affected sites. Soil air permeability, moisture content, potential, bulk density, porosity and clay content were measured on the vertical face of the soil pit at depths of 0-0.15 m, 0.15-0.3 m, 0.3-0.45 m, 0.45-0.6 m, 0.7-0.85m and 0.95-1.1 m.

Soil drainage in healthy and dry root rot affected sites was monitored over several irrigation cycles using a neutron moisture meter in the MIA. Piezometers were used to monitor the water table on the Central Coast of NSW.

RESULTS AND DISCUSSION

The soil types examined in the MIA can be classified as red-brown earths, with a transition to brown cracking clays. These soils are often susceptible to waterlogging. Results indicate that sites where dry root rot is occurring are subject to soil compaction, poor soil aeration and temporary waterlogging in the subsoil (0.45-1.1 m). Soil air permeability was significantly higher ($p<0.05$) at healthy sites (30.32 μm^2 cf. 5.44 μm^2), as was air-filled porosity. Measured air-filled porosities were low for all sites, with the diseased sites lower than the generally accepted critical value of 10% (11.14% cf. 8.59%). As measurements were obtained mid-way through an irrigation cycle, the amount of soil air available to roots is likely to be substantially less for several days after irrigation or in periods of heavy rainfall. Soil moisture content (0.22 g/g cf. 0.25 g/g), and water-filled porosity (31.79% cf. 35.59%) were significantly higher at diseased sites, but soil moisture potential (-0.97 MPa cf. -0.1 MPa) and pore size (4.09 µm cf. 4.5 µm) were not significantly different ($p>0.05$). This suggests that all sites have the same average soil pore size, but the diseased sites have a greater number of pores filled with water. This would have contributed to the poor aeration and drainage of the dry root rot sites. Bulk density and clay content were not significantly different, though average bulk density (1.49 g/cm^3) of all sites was high for the amount of clay (45%) if optimal root growth is to be obtained. No significant differences were found above 0.45 m.

Results from the neutron moisture meter indicated that where dry root rot occurred, the soil was subject to higher soil moisture contents (cf. healthy sites), and thus lower air availability for plant roots. In addition, water sat on the soil surface for longer after irrigation or rainfall, and the soil was slower draining.

Soil on the Central Coast is generally a sandy yellow earth and considered to be free draining. Installation and monitoring of piezometers showed that after periods of heavy rainfall, a perched watertable appeared within the root zone of the affected areas (within 0.45 m of the soil surface cf 0.8 m in healthy areas) due to submerged ironstone boulders and/or an impermeable clay layer at depth. In high watertable areas, dry root rot occurred.

Previous literature suggests that dry root rot is associated with temporary waterlogging and poor soil aeration. The study reported here suggests the same association. Further investigation into the contributory role of *C. micaceus* and *Fusarium spp.* to dry root rot, under conditions of temporary waterlogging is currently underway.

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THE INFLUENCE OF CARBENDAZIM ON MYCOTOXIN PRODUCTION IN *FUSARIUM SPOROTRICHIOIDES*

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ABSTRACT

Carbendazim was shown to influence production of T-2 toxin by *Fusarium sporotrichioides* maintained at 25°C, but in isolates transferred from 25°C to 11°C, both T-2 toxin and zearalenone production was reduced as carbendazim concentration rose.

INTRODUCTION

Mycotoxins are possibly the most unfamiliar and least investigated of the natural products that affect man and animals. There is an increasing awareness that this structurally diverse group of naturally occurring fungal toxins is being implicated in toxic syndromes. Such toxins are primarily to be found in agricultural crops such as cereals and oilseeds and products derived from them (Smith *et al.*, 1994).

Fusarium species are widely acknowledged to be phytopathogens of cereals. These fungi are also recognised as producers of mycotoxins including zearalenone (ZEN), T-2 toxin (T-2) and neosolaniol (NEO). Effective fungal disease control strategies require the use of fungicides, but they can influence mycotoxin production, their effects being variable and dose-dependent (D'Mello *et al.*, 1996).

Little attention has been given to the range of environmental factors and fungicides which might allow growth and accumulation of mycotoxins in grains.

The aim of this experiment was to investigate, in factorial combination, the effects of time, temperature and fungicide level on mycotoxin production in pure cultures of *Fusarium sporotrichioides*.

MATERIALS AND METHODS

Fusarium sporotrichioides 309349 from IMI was used. Peripheral plugs from colonies of this culture were used to prepare 5d old cultures. A plug from the 5d old culture was placed under aseptic conditions onto the centre of each petri dish containing PDA with different levels of fungicide. Carbendazim (Bavistin, BASF) was used. The concentration of the active ingredient was 50%. The fungicide was dissolved in ethanol and incorporated in the sterilized medium to provide carbendazim concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 µg/ml. The solvent without fungicide was added to the control. The cultures were incubated at 25°C for 5d, then half of the replicates were transferred to 11°C. At 6, 14 and 26d, colonies were

extracted with 20 ml chloroform. Filtered extracts were reduced in volume with a rotary evaporator and finally dried under N2 prior to storage at -20°C. For the determination of toxins, the extracts were resuspended in 0.1 ml chloroform and spotted on TLC plates. The plates were then developed in TEF (toluene: ethyl acetate: formic acid, 5:4:1) and examined under UV light. The presence of mycotoxins was evaluated visually by comparing the R_f and colour with the appropriate standards and quantified by densitometry. Analysis of variance for a factorial design was carried out using minitab and significant differences established.

RESULTS AND DISCUSSION

Production of ZEN, T-2 toxin and NEO was significantly influenced by time ($P<0.001$), whereas temperature ($P<0.01$) and concentration of fungicide ($P<0.05$) induced significant effects only for ZEN and T-2 toxin synthesis. However, only the main effects of fungicide concentration and temperature are presented in Table 1. At 5 µg/ml, carbendazim significantly ($P<0.05$) increased T-2 toxin production in cultures maintained at 25°C. On the other hand, in cultures in the 25-11°C regime, T-2 toxin production was significantly ($P<0.05$) reduced by carbendazim at 5 µg/ml, whereas ZEN production was reduced by the fungicide at levels of as low as 1 µg/ml ($P<0.05$).

Table 1. The effect of temperature regime and carbendazim application on mycotoxin production in cultures of *Fusarium sporotrichioides*

Carbendazim level (µg/ml)	Temperature regime					
	25°C			25 - 11°C		
	Mycotoxin (µg/ml of culture extract)					
	ZEN	T-2	NEO	ZEN	T-2	NEO
0	0.31	18.5	6.7	0.65	21.8	10.7
1.0	0.26	13.3	5.8	0.28	15.7	5.0
2.5	0.09	18.0	5.6	0.54	15.9	5.4
5.0	0.26	29.8	13.4	0.31	10.0	4.2
7.5	0.15	18.0	10.9	0.51	9.7	4.7
10.0	0.04	15.9	3.6	0.04	2.9	1.1
Significance	NS	<0.05	NS	<0.01	<0.05	NS
SEM (df=35)	0.091	3.30	2.68	0.091	3.30	2.68

ZEN= zearalenone; T-2 = T-2 toxin. NEO= neosolaniol

Taken together with previous results (D'Mello *et al.*, 1996) it is concluded that T-2 toxin production is increased with carbendazim application. In contrast, ZEN production is reduced with this fungicide

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INFECTION OF POTATO BY *VERTICILLIUM DAHLIAE* UNDER CONTROLLED ENVIRONMENT CONDITIONS

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ABSTRACT

The extent to which *Verticillium dahliae* causes a problem in the UK potato crop is unknown. Three UK isolates of *V. dahliae*, two from potato and one from linseed, were used to root-dip inoculate two-week-old potato plantlets (cv. Record). Inoculated plants were grown in controlled environment (CE) room conditions. All isolates were found to infect the potato plants and all were able to induce wilt symptom expression under CE conditions.

INTRODUCTION

Verticillium dahliae is one of several species of fungi causing vascular wilts. The pathogen has a wide host range and is known to cause disease on many economically important crops including cocoa, cotton, linseed and tomato. When it infects potato (*Solanum tuberosum*), along with other interacting factors the resulting disease is often called Potato Early Dying (PED). Symptoms of this syndrome closely resemble those of natural senescence, with an acropetal progression of chlorosis and necrosis. Actual wilting of green petioles is not always seen. The disease can result in a significant loss of yield. Up to 30 - 50 % losses have been reported in regions of North America where the disease on potatoes has been well documented (Powelson & Rowe, 1993).

The extent to which *V. dahliae* causes a problem in the UK potato crop is not known. Surveys and trials run by ADAS are currently evaluating PED in the field. This study aims to assess the pathogenicity of different isolates of the fungus, and the susceptibility of currently recommended UK cultivars to infection, in controlled conditions.

MATERIALS AND METHODS

Potato cultivar Record, which has developed PED symptoms in field trials, was used. Potato eyes were removed from seed tubers using a melon baller and were allowed to sprout in vermiculite for two weeks. After this time, plantlets were uprooted, excess vermiculite was gently rinsed off under a tap and they were then root dipped in conidial inoculum ($1x10^8$ conidia/ml) of one of three isolates of *V. dahliae* (Table 1) for one hour. Inoculated plants were re-potted in two-litre pots in a loam based soil and were arranged in a randomised block design (five replicates) in a CE room (23°C 10h day; 18 °C night).

Plants were assessed weekly for stem height and disease development (% wilt). After 10 weeks, stems were excised at the base. Sections were sampled from each stem, plated out

on antibiotic-amended agar and observed for growth of *V. dahliae* from the vascular bundles five days later. Stem sections were also used in a maceration-dilution assay to semi-quantify the degree of colonisation.

RESULTS AND DISCUSSION

Table 1. Height, disease expression and colonisation of *V. dahliae* of potato plants (cv. Record) 10 weeks after inoculation with isolates of *V. dahliae*.

Isolate	Original host	Height of potatoes (cm)	% Wilt	Re-isolation of *V. dahliae* Yes:No	Concentration of *V. dahliae* in stem (propagules/g fresh weight tissue)
5397	Potato	41.2 a†	36.0 a†	5:0	6.9×10^2 a*
5399	Potato	48.2 b	63.0 b	5:0	5.8×10^3 a b
Lin1-a	Linseed	39.8 a	65.0 b	5:0	1.9×10^4 b
Control		46.3 a b	9.4 c	0:5	0 c

† ANOVA was used (p=0.05). Values followed by a different letter are significantly different.
* Values represent back-transformed means. ANOVA was carried out on log-transformed data.

The re-isolation data confirmed that *V. dahliae* colonised the host (Table 1). There was no significant difference between the heights of infected and control plants, suggesting that under CE room conditions, stunting is not a symptom of *V. dahliae* infection. There was a significant difference (p=0.05) between the % wilt symptoms of the inoculated plants compared to the control plants. This indicates that under these CE conditions potato plants can be induced to express typical wilt symptoms as seen in the field. The concentration of *V. dahliae* propagules in the host did not appear to correlate with the severity of the wilt observed. For example, although there is no significant difference between the average concentration of the two potato isolates in the host, there is a significant difference (p=0.05) between the degree of wilt expression induced by these two isolates.

It is also interesting to note that the linseed isolate was able to induce severe symptoms of wilt comparable to one of the potato isolates. The linseed isolate was found to have colonised the host to a significantly greater extent (p=0.05) than one of the potato isolates. This confirms that isolates of *V. dahliae* are able to infect a host different to that from which it originated. This cross-infection may have important consequences for the epidemiology of wilt diseases such as PED.

ACKNOWLEDGEMENT

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RELATIONSHIP BETWEEN PLANT MORPHOLOGY AND SEVERITY OF FUSARIUM EAR BLIGHT IN EIGHT CULTIVARS OF WINTER WHEAT

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ABSTRACT

In order to determine the significance of morphological characters of winter wheat on the severity of *Fusarium* ear blight,a field trial was set up in 1994/95 to examine the following characters; height, peduncle length, compactness of ear and angle of flag leaf. Although taller varieties such as Spark and Cadenza showed reduced severity of ear blight compared with shorter cultivars such as Brigadier and Genesis no other morphological character showed any significant correlation with disease severity.

INTRODUCTION

Ear blight caused by a range of *Fusarium* species including *F.culmorum, F.graminearum, F.avenaceum, F.poae* and *Microdochium nivale* is an important disease of wheat. As well as causing significant yield losses under certain conditions, the fungus can synthesize mycotoxins which adversely affect both humans and animals. Most cultivars of winter wheat (*Triticum aestivum*) are susceptible to the pathogens whilst some cultivars have limited resistance. No complete resistance to these pathogens has been observed (Snijders, 1990)

Recent observations at Harper Adams indicated that several features of wheat cultivars may be associated with resistance. This trial aimed to reveal morphological features that could be associated with resistance under artificially inoculated conditions.

METHOD

During the 1994/95 season morphological characters which might affect the micro-climate around the ear were measured in eight cultivars of winter wheat. These included the taller cultivars Spark, Cadenza, Mercia and Riband and the shorter cultivars Hereward, Brigadier, Hunter and Genesis. Total plant height, length of peduncle, angle of flag leaf and compactness of the ear were assessed on tagged plants grown in 2m x 1m uninoculated plots. Other plots were artificially inoculated with a mixture of *Fusarium* spp (*F.culmorum, F.graminearum, F.avenaceum* and *Microdochium nivale*) at a concentration of 2.5×10^5 ml before being mist irrigated to encourage disease development. The percentage of spikelets showing necrosis on individually tagged ears 4,5 and 6 weeks after inoculation was then assessed.

RESULTS

After 6 weeks there were significant differences in disease severity and morphological features between cultivars. There was a significant negative correlation between plant height and severity of *Fusarium* Ear Blight (r=-0.572) (Table1). The taller varieties Spark and Cadenza were more resistant than the shorter varieties such as Genesis and Brigadier.

Table 1: Relationship between ear blight and morphological characters of eight cultivars of winter wheat six weeks after artificial inoculation with a mixture of *Fusarium* species and *Microdochium nivale*.

	Tiller height (cm)	Compactness of ear (Number of spikelets/cm of rachis)	Length of peduncle (cm)	Angle of flag leaf (degrees from the vertical)
Correlation coefficient between traits and %Ear blight	-0.572**	-0.185	-0.015	0.226

**Significant at 1% level

Six weeks after inoculation Spark showed 25% ear blight and Cadenza 28%. In Genesis and Brigadier the figures were 55 and 58% respectively. No significant associations between any other morphological character and disease severity were observed.

DISCUSSION

Results from this trial show that the tall cultivars Spark and Cadenza were less severely affected by *Fusarium* ear blight than the shorter cultivars. Results from a naturally infected trial reported by Mesterhazy (1995) showed that disease severity was 50% less on taller cultivars (above 1m) than dwarf cultivars (below 70cm). Mesterhazy speculated that these differences were due to the proximity from the initial source of infection in the debris on the soil surface. However the data shown in this work from an artificially inoculated trial suggest that factors other than proximity from the source of infection may be involved. Studies are underway to examine the possible relationship between microclimate, disease severity and height in a range of cultivars of differing heights.

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THE TOXICITY OF FOUR TROPICAL PLANT EXTRACTS TO ARTHROPOD PESTS OF MEDICAL AND AGRICULTURAL IMPORTANCE

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ABSTRACT

Problems with insecticides and acaricides in current use, including resistance and environmental toxicity, have required the investigation of new products for pest control. This paper describes the results of bioassays with four tropical plant extracts and compares their toxicity to the two-spotted spider mite (*Tetranychus urticae*), the peach-potato aphid (*Myzus persicae*), the Yellow Fever mosquito larvae (*Aedes aegypti*), the cotton leaf worm (*Spodoptera littoralis*), and the European house-dust mite (*Dermatophagoides pteronyssinus*).

INTRODUCTION

In recent years agrochemical companies have turned to native cultures in their search for new pesticides (Benner 1993). The research described in this paper involved assaying extracts of four plants to determine whether they were toxic to a variety of arthropod pests. The pest species chosen for the bioassays included world-wide major crop pests and two pest species of medical importance.

MATERIALS AND METHODS

Plant extracts for the bioassays were prepared using cold ethanoic extraction. The concentration values used, which are for bulk plant material (mg) in ethanol (ml), ranged from 0.4-500mg/ml. Control treatments comprised the ethanol solution alone. Pest species were obtained from cultures maintained by the University of Paisley. Various bioassays were performed depending upon the species tested i.e. leaf-dip tests for the spider-mites, topical application for the *Spodoptera*, spray application for the aphids, immersion and fabric tests for the dust mites, and larval bioassays for the mosquitoes (McDonald & Tovey 1993, Jepson 1993). In each bioassay a minimum of four replicates per treatment was used. The data are described in terms of the maximum percentage mortality obtained with each of the extracts evaluated.

RESULTS AND DISCUSSION

Results of the bioassays are given in Table 1 for plants 1 and 2 and in Table 2 for plants 3 and 4. The tables show the concentration of plant material that gave maximum mortality at 7 days

after treatment (24 hours for *D. pteronyssinus*). Where there is no figure given the mortality obtained using the extract was not significantly greater than the control treatment. The house-dust mite was susceptible to all four plant extracts, the spider-mites to three plant extracts, the *Spodoptera* to two plant extracts and the aphids and mosquito larvae to one plant extract.

Table 1. The extract concentration of plants 1 & 2 and it's associated toxicity to the pest species assayed.

Pest Species	Plant 1		Plant 2	
	% Max. Mortality	Conc. mg/ml	% Max. Mortality	Conc. mg/ml
T. urticae	100	80	-	-
M. persicae	-	-	-	-
S. littoralis	-	-	100	500
A. aegypti (larvae)	34	200	-	-
D. pteronyssinus	74	0.4	58	0.4

Table 2. The extract concentration of plants 3 & 4 and it's associated toxicity to the pest species assayed.

Pest Species	Plant 3		Plant 4	
	% Max. Mortality	Conc. mg/ml	% Max. Mortality	Conc. mg/ml
T. urticae	100	40	100	20
M. persicae	-	-	93	20
S. littoralis	40	40	-	-
A. aegypti (larvae)	31	400	100	400
D. pteronyssinus	60	4	80	400

Our results show that many of the extracts evaluated were toxic to the pest species assayed. The next stage in our research will be to use Soxhlet extraction and HPLC to try to isolate the active component(s). Our ultimate goal will be to develop these extracts into commercially useful chemicals for pest control.

ACKNOWLEDGEMENTS

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INFLUENCE OF PLANT GENOTYPE AND PHYSIOLOGICAL ACTIVITY ON THE DISTRIBUTION OF PESTS IN BRASSICA CROPS

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ABSTRACT

The low diversity, uniform quality and high productivity of monoculture crops increases the likelihood that pests locate foodplants. This study demonstrates the differential distribution of pests between cabbage (*Brassica oleracea*) cultivars treated or not treated with insecticides. Pest densities are related to plant physiological attributes such as photosynthetic and transpiration rates.

INTRODUCTION

The concentration of pests in crop environments is a consequence of the abundance of hostplants and the ease with which they are located (Cromartie, 1981). Predators and pests are influenced by the diversity of crops and adjacent field areas (Wratten & Thomas, 1990). The morphology and physiology of cabbage cultivars can differ markedly. Structural diversification of the crop can be increased by growing a variety of cultivars simultaneously. Physiological characteristics of plants influence herbivory as they are both influenced by changes in leaf water levels, nutrient content and cuticle thickness (Mattson & Haack, 1987). This study examines the physiological activity and the folivorous invertebrate fauna of three cultivars of cabbage.

MATERIALS AND METHODS

Foliar surveys were conducted bi-weekly on 192 plants of the cultivars Duncan F1 and Green Express F1 (25.5.95 - 19.7.95) and Slawdena and Green Express F1 (19.7.95 - 10.10.95) at Barton Moss Farm, Irlam, UK. Plants were treated with demeton-S-methyl and cypermethrin. Photosynthetic rate (A), transpiration rate (E) and stomatal conductance (Gs) were measured using an infra red gas analyser (LCA3; Analytical Development Company, Hoddesdon, UK).

RESULTS AND DISCUSSION

Lepidopteran larvae, almost absent from site one, were unevenly distributed in site two. The head forming Green Express supported more *Plutella xylostella* and *Pieris rapae* larvae (Table 1). The latter were found at higher densities on plants with higher A, E and Gs (Table 2). The authors have evidence to suggest egg-laying *P.rapae* oviposit more often on plants with higher photosynthetic rates. In site one the highest densities of aphids (*Myzus persicae* and *Brevicoryne brassicae*) were found on the flat packing cultivar Duncan F1 which provided shelter in its tightly curled leaves. Green Express supported more than the smaller head forming Slawdena. No physiological factor correlated with aphid densities. More parasitic wasps, including parasitoids of aphids and lepidoptera, were found on cultivars which supported higher numbers of pests.

Table 1. Comparison of the mean number of invertebrates (± standard error) on untreated cultivars using rank means tests. Calculated from whole season data (* indicates mean is significantly higher at $p < 0.05$ level).

	Duncan	Green Express	Slawdena	Green Express
	Site One		Site Two	
Myzus persicae	1.89 (±0.37)*	0.73 (±0.22)	0.03 (±0.02)	0.16 (±0.12)*
Brevicoryne brassicae	1.74 (±0.52)*	1.26 (±0.29)	0.53 (±0.19)	3.78 (±3.20)*
Pieris rapae	0.12 (±0.08)*	0.01 (±0.01)	0.20 (±0.04)	0.31 (±0.15)
Plutella xylostella	0.12 (±0.08)	0.11 (±0.06)	0.61 (±0.10)	1.32 (±0.46)*

Table 2. Spearman rank correlations between peak animal densities and plant physiological activity (* denotes siginificance at $p < 0.05$ level, ** denotes significance at $p < 0.005$ level, NS = not significant).

	Photosynthetic Rate (A)	Transpiration Rate (E)	Stomatal Conductance (Gs)
Myzus persicae	NS	NS	NS
Brevicoryne brassicae	NS	NS	NS
Plutella xylostella	NS	NS	NS
Pieris rapae	$r_s = 0.344$**	$r_s = 0.234$*	$r_s = 0.248$*

CONCLUSIONS

Diversification of the crop was achieved by growing cultivars with structural and physiological differences. Pests and potentially beneficial organisms were differentially distributed between these genotypes. Plant physiological attributes were related to the number of *P.rapae* larvae which may result from adult egg-laying behaviour. It is suggested that the combination of genotypes grown together influences folivorous faunal communities and that plant physiological measurements may contribute to the understanding of the relationships between pests and crops.

ACKNOWLEDGMENTS

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CAUSES OF EARLY POPULATION CRASHES OF THE PEA APHID, *ACYRTHOSIPHON PISUM*, ON PEA CROPS

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ABSTRACT

The method used by ADAS to provide spray forecasts for pea aphid control on combining peas results in unnecessary pesticide applications on 30% of occasions because populations crash unexpectedly early. The effects of temperature and plant growth stage on aphid mortality and behaviour were examined to explain these early declines. Older plants and higher temperatures resulted in greater mortality and emigration. It is hypothesised that these population crashes are caused by the interaction of high temperature with more advanced plant growth stages.

INTRODUCTION

Populations of the pea aphid frequently cause economic damage to both combining and vining pea crops, which they colonise every spring. ADAS currently advise that combining crops be sprayed if 20% or more of shoots become infested at any time between flowering and pods becoming fully formed on the fourth truss (Lane & Walters, 1991). The drawback to this forecasting system is that pea aphid populations can crash to virtually zero at growth stages of the pea crop where they usually remain high. Walters *et al.* (1994) estimated that this resulted in recommendations for unnecessary insecticide applications on 30% of occasions. Biddle *et al.* (1994) also noticed these early population declines on vining pea crops. This work examines to what extent high temperature and crop growth stage can account for these premature population decreases.

MATERIALS AND METHODS

Eighteen newly flowering and 18 two week old pea plants were placed in Latin squares in 2 growth cabinets, one at 15°C and one at 25°C. Twenty newly born pea aphid nymphs were placed in a small cone at the base of each plant, which was then surrounded with an acetate cylinder to prevent the aphids emigrating. After 8 days the surviving aphids were counted. The experiment was repeated without acetate cylinders, so that aphids were free to leave the plants.

Plants were grown in a greenhouse using Levington multi purpose compost and nymphs were produced at 20°C on pea plants.

RESULTS

Analyses of variance showed that when the aphids were prevented from leaving plants by acetate cylinders, mortality was significantly higher on older plants and at higher temperatures,

although there was no interaction between the two factors ($P<0.001$, $P=0.011$ and $P=0.278$ respectively). See Figure la.

In the second experiment, where aphids were free to leave the plants, temperature and plant growth stage interacted ($P<0.001$) and no aphids remained on the older plants after 8 days. See Figure lb.

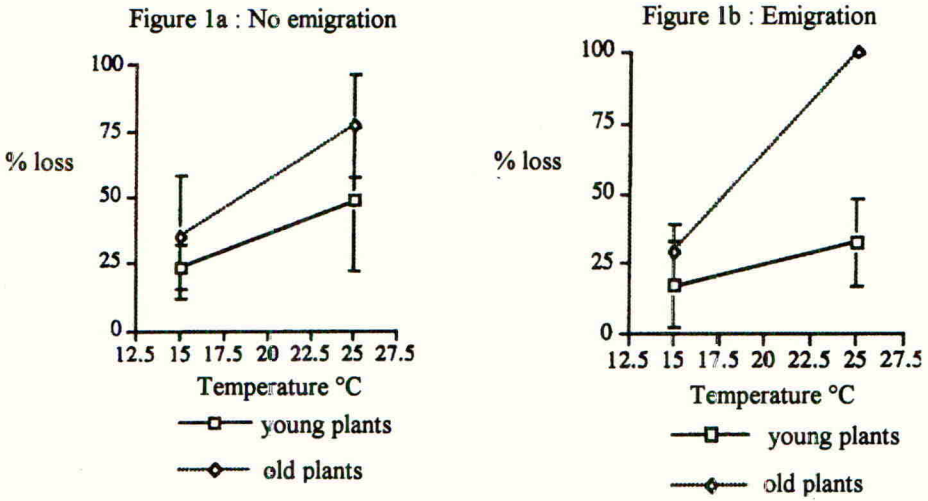

Figure 1 Percentage loss of aphids after 8 days on young and old pea plants at 15°C and 25°C. Error bars show mean ± standard error.

DISCUSSION

It seems probable that temperature and crop growth stage interact in the field, resulting in population crashes of the pea aphid through a combination of increased mortality and emigration.

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POTENTIAL APHID PESTS OF THE BIOMASS CROP *MISCANTHUS*

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ABSTRACT

The reproductive activity of two aphid species, the bird cherry-oat aphid *Rhopalosiphum padi* and the corn leaf aphid *Rhopalosiphum maidis* was investigated on three growth stages of the biomass crop *Miscanthus sinensis*. Only *R. maidis* were found to reproduce successfully. The RPV isolate of barley yellow dwarf virus (BYDV) was successfully transmitted to *M. sinensis* by *R. maidis* and significantly reduced the biomass potential of infected plants. The results are discussed considering the physiology and origin of the host plant and the distribution and native range of the aphid species tested.

INTRODUCTION

The promising biomass yields being attained from field trials of the oriental, C_4 grass *Miscanthus sinensis* (Kilpatrick *et al.*, 1994) indicate the likelihood that this crop will play a significant role in future agriculturally based European energy programmes. The majority of research has concentrated on the expected yields, physiology and cultivation practices of this newly domesticated plant genus. Little concern has been paid to the economically important insect pest complex that is likely to develop once it is adopted at a large scale commercial level. It is the aim of this study to assess the host plant suitability of *M. sinensis* to two European aphid pest species, the bird cherry-oat aphid, *Rhopalosiphum padi* and the corn leaf aphid, *Rhopalosiphum maidis*.

MATERIALS AND METHODS

Individual apterous adult aphids were caged on two and five leaf *M. sinensis* seedlings, mature rhizomatous *M. sinensis* 'Giganteus' plants and three weeks old barley seedlings, allowed to give birth to nymphs for 24 h and then removed along with all but the fifth nymph. These were then each reared to maturity with the time from birth to first reproduction (pre-reproductive time, *d*) and the number of progenies (*Md*) produced in an equal time to *d* being recorded. The intrinsic rate of natural increase (*rm*), as formulated by Wyatt & White (1977), was then calculated to compare the effect of each plant growth stage on the reproductive activity of both aphid species.

Five adult apterous viruliferous aphids, fed on positively tested BYDV infected oat plants for 48 h, were caged on previously uninfested six weeks old *M. sinensis* seedlings. After 72 h all aphids were removed from the plants using the selective aphicide 'Aphox'. Plants were held in an aphid free environment for a further six weeks when symptom expression was noted and the presence of BYDV detected using a standard enzyme-linked immunoabsorbent assay (ELISA) following the protocol of Lister & Rochow (1979). Above soil plant material of each plant was then dried at 72°C for 48 h and subsequently weighed to compare the effect of BYDV on biomass

accumulation between infected and uninfected plants. The experiment used oat plants infected with the RPV isolate of BYDV for *R. maidis* and the PAV isolate for *R. padi*. All experiments were conducted at 20°C and a photoperiod of 16 h.

RESULTS AND DISCUSSION

Only *R. maidis* were found to reproduce successfully on all three growth stages of *Miscanthus* (Table 1) indicating that this novel crop species is a suitable host plant. Reproductive activity was generally higher on later growth stages but lower overall than on the barley. The ability of this species to transmit the RPV isolate of BYDV to *Miscanthus* and the significant decrease in biomass yield (Table 2) resulting from infection, clearly shows the potential severity that this species could have on future commercial plantations. The inability of *R. padi* to reproduce on *Miscanthus* is most like due to the inability of this species to penetrate the comparatively much thicker epidermis and bundle sheaths present in C_4 grasses (Weibull, 1990). *R. padi* is palaeartic in origin and is therefore adapted to feeding on the predominantly C_3 plants found in this region as opposed to the Asiatic originating *R. maidis* that is more commonly found feeding on C_4 hosts.

Table 1. Mean relative intrinsic growth rate (*rm*) of apterous *R. maidis* on three growth stages of *M. sinensis* and barley. Letters in common differ significantly at the $p < 0.05$ level.

Treatment	n	*rm*
2 leaf	11	$0.254^{A,B,C}$
5 leaf	10	$0.288^{A,D}$
rhizomatous	12	$0.288^{B,E}$
barley	10	$0.360^{C,D,E}$

Table 2. Comparison of RPV infected *M. sinensis* with healthy uninfected controls. Letters in common differ significantly at the $p < 0.05$ level.

Treatment	n	*Biomass* (mg)
Infected	8	0.471^{F}
Uninfected	18	0.611^{F}

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FITNESS COSTS ASSOCIATED WITH PYRETHROID RESISTANCE IN *HELIOTHIS VIRESCENS*

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ABSTRACT

Fitness studies where carried out on monogenically resistant isogenic strains of *Heliothis virescens*, developed by backcrossing insecticide resistant with susceptible strains. Reductions in fitness are dependent on the mechanism of resistance. Target site insensitivity leads to a large loss of fitness whilst enhanced metabolism leads to a small loss of fitness. These reductions occur in all measured developmental parameters, and in the intrinsic rate of increase. A simple model estimates the time taken for a reduction from 90% to 10% resistance in the two strains as 78d for Target Site Insensitivity and 162d for Enhanced Metabolism.

INTRODUCTION

Management strategies for combating pesticide resistance may depend on reduced fitness in resistant as compared to susceptible individuals. Only then will susceptibility return and efficacy of the pesticide be restored. Any species which is not panmictic will have a range of biotypes (Birch *et al.*, 1962), so it is essential to study fitness in isogenic strains where resistance is the only variable between strains. A range of resistance mechanisms occur, often in the same species. As fitness costs will be related to the mechanism, fitness studies have to be carried out together with mechanistic studies.

MATERIALS AND METHODS

A metabolically resistant strain (EMR) was developed by backcrossing MS2 (metabolically resistant strain bred at Reading) with the susceptible strain (SUR) six times, and subjecting the neonates to a discriminating dose each generation. The target site insensitive strain (NIR) was developed by a backcross of NI1 (target site insensitive resistant strain bred at Reading) to SUR followed by a test cross and discriminating dose. Target site insensitivity was confirmed by neurophysiological analysis (McCaffery *et al.*, 1995). Levels of cypermethrin tolerance were estimated during the experiment by first instar foliar residue bioassays. Fitness was determined using replicated cohorts of 170 neonates, recording mortality and development time from oviposition to emergence. Fecundity was determined on a random selection of 50 female pupae, pair mating with males emerging not more than two days after the female. Eggs were collected every two days until the female reached 16 days old, and neonate numbers counted. The intrinsic rate of increase (r_m) was calculated using the equation $\sum(ke^{-r_m t})=1$ (Dobzhansky *et al.*, 1964) (k=daughters per female, r_m=intrinsic rate of

increase, t=time from oviposition to oviposition) in an iterative process carried out using Microsoft Excel. Modelling the time taken for a reduction from 90% to 10% resistance uses the equation $dN/dt=e^{r_m}N$ (Dobzhansky *et al.*, 1964) (N=population size).

RESULTS

Table 1 shows that target site insensitivity confers low levels of resistance at high cost so that resistance would rapidly decrease upon the cessation of pesticide use. In contrast, metabolic mechanism(s) confer much higher resistance with lower, although still significant, costs, and more than twice the reversion time. In spite of this, target site insensitivity often occurs first in the field suggesting that this mutation occurs more frequently than that for enhanced metabolism which may only occur under the protection of pre-existing resistance.

Table 1. Fitness parameters for resistant and susceptible *H.virescens* (95% Confidence Limits)

	SUR	**EMR**	**NIR**
Cypermethrin LD_{50} (ppm)	1.64 (1.34-1.97)	147 (107-210)	22.9 (13.9-37.8)
Cypermethrin LD_{90} (ppm)	7.1 (5.4-10.5)	1547 (792-5379)	196 (90-1373)
Resistance ratio at LD_{50}	-	89.4	13.9
Time from oviposition to emergence (days)	28.10 (±0.19)	28.81 (±0.13)	32.03 (±0.25)
% survival (oviposition to oviposition)	64.8 (±10.1)	51.5 (±8.9)	53.7
Fecundity	746.6 (±44.6)	541.3 (±52.1)	316.7 (±60.8)
Intrinsic rate of increase	0.158 (±0.004)	0.131 (±0.007)	0.101 (±0.010)
Number of replicates	5	6	2
Numbers of pairs	169	172	63
Days for reversion of resistance from 90 to 10%	-	162	78

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EFFECT OF HOST PLANT VOLATILES ON THE FLIGHT BEHAVIOUR OF *PIERIS RAPAE*

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ABSTRACT

The behaviour of female *Pieris rapae* was tested in a wind tunnel in the presence of host plant volatile odour cues. The results show females flew more often and moved upwind in response to such cues.

INTRODUCTION

Host plant volatiles have been shown to be important cues affecting the orientation of many phytophagous insects. Previously no evidence was available for the role host volatile chemicals play in the orientation of *P. rapae* to its host plant (Renwick & Radke, 1988). In this study we investigated the effect of host plant volatiles on the frequency and duration of flight behaviours and the positioning of female butterflies in a wind tunnel relative to the odour source.

METHODS

Plant volatiles were collected as described in Robertson *et al.* (1993). Solvent elution was used to collect volatiles trapped onto Tenax-ta (mesh size 60/80) from cabbage var. Golden Acre with the final concentration of volatiles being 1.0 gram leaf equivalents. Ten laboratory-reared adult females were grouped and tested twice as one of three replicates. Independent groups were tested in each of the other two replicates. The wind tunnel was 2.0m wide, 1.75m in length and 1m high and illuminated by eight evenly spaced fluorescent tubes providing full spectrum light. Wind tunnel temperature was approximately 22 °C and wind speed was 1 m/s. Females were introduced and allowed to acclimatise for 30 min. Three vials of test chemical (solvent or volatiles (volume 60 ml)) were placed 0.50 m apart across the width of the tunnel. The vials were introduced 25 min into the acclimatisation period. After the full 30 min of the acclimatisation period was complete, behaviours were recorded at 10 min intervals for 90 min duration. The behaviours recorded were the positions of the butterflies i.e. upwind (0-0.58 m); middle (0.58-1.16 m) and downwind (1.16-1.75 m) of the wind tunnel and their activity (flight, rest, feeding).

RESULTS AND DISCUSSION

The typical behaviour of a female in the wind tunnel can be described as resting interspersed with flutter bouts. The flutter bouts are characterised by flight upwind with casting across the wind tunnel and rapid flights downwindTable 1 shows the mean of the behaviours recorded and standard error of the mean for the three replicates. A Chi-square test (Chi-sq=69.985; df=6; $p>0.001$) indicated highly significant differences between the behaviours for volatile and solvent treatments. Female *P. rapae* showed an increase in the number of butterflies in flight,

particularly in the upwind and middle sections when exposed to host plant volatiles as compared to the ether control. More butterflies rested in the upwind section and lesser numbers rested in the middle and downwind sections when exposed to volatiles as compared to ether. There appears to be no effect on feeding behaviour.

The results from this work show that in the presence of host volatiles, females exhibited a greater propensity towards flight and moved towards the source of the volatiles as compared to solvent controls. These results are largely in agreement with the findings of Aluja *et al.* (1993) for *Rhagoletis pomonella* which flew more often in air permeated with host plant volatiles. Evans (1991) found that *Dasineura brassicae and Ceutorhynchus assimilis* showed an upwind movement and significantly fewer were found in the downwind section of the wind tunnel.

These results indicate that volatile chemicals emanating from the host plant do influence the searching behaviour of female *P. rapae*. The pre-alighting search for hosts appears to involve both olfactory and visual cues, with the prominent cue being visual (Renwick & Radke 1988). Olfactory cues act at a greater distance bringing a female within range of the host plants for visual discrimination to occur.

Table 1. Mean number (± SE) of butterflies in each behavioural category, averaged across times and replicates.

Behavioural Class	Volatiles	Ether
Feeding	0. 0.3 ± 0.07	0.33 ± 0.07
Flying Upwind	1.42 ± 0.14	0.42 ± 0.08
Flying Middle	1.48 ± 0.16	1 ± 0.16
Flying Downwind	0,78 ± 0.1	0.53 ± 0.09
Resting Upwind	1.6 ± 0.15	1.23 ± 0.14
Resting Middle	3.2 ± 0.24	4.92 ± 0. 27
Resting Downwind	1.2 ± 0.13	1.55 ± 0.16

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THE ISOLATION OF FUNGAL PROTOPLASTS FOR STUDIES OF FUNGICIDE ACTION

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ABSTRACT

Physiologically active protoplasts have been isolated from *Botrytis cinerea*, *Phytophthora infestans* and *Septoria nodorum*, and viability assessed using fluorescent dyes and the measurement of respiration.

INTRODUCTION

First isolated in the late 1950 s, fungal protoplasts have been increasingly used to study cell processes such as cell wall regeneration, enzyme secretion, macromolecule synthesis and membrane fusion (reviewed by Isaac 1985). Once established, characterised protoplast systems can be used to investigate the effects of fungicides upon fungal cell processes.

MATERIALS AND METHODS

Strains and media

Field isolates of *B. cinerea* were grown on potato dextrose agar (Oxoid). Sporulating cultures were obtained by incubation of 4 d old dark-grown colonies under near UV light for a further 3-4 d. Cultures of *P. infestans* were grown on rye meal agar (g/litre: rye meal 12.5, glucose 1.25, $CaCO_3$ 10, agar (Fisons) 20, V8 juice 50 ml). Sporangia were produced by cultures grown in the dark for 14 d. Cultures of a wheat adapted wild-type strain of *S. nodorum* (BS171) were grown on CzV8 medium (Cooke & Jones 1970). Sporulating cultures were obtained by incubation of 5d old dark-grown colonies under near UV light for a further 8-10 d.

Protoplast isolation

B. cinerea: 100 ml of a $2x10^5$ spores/ml suspension in glucose growth medium, were incubated in 250 ml Erlenmeyer flasks at 20°C for 24 h on a reciprocal shaker, 100 rpm. Mycelia were harvested by filtration through a sterile 20 µm sieve, washed with sterile 0.8 M KCl buffered to pH 5.8 with a 0.1 M phosphate buffer (Phos-KCl), and resuspended in the same buffer containing 5 mg/ml lysing enzymes from *Trichoderma harzianum* (Sigma). After incubation at room temperature for 2 h, the protoplasts were harvested by filtration through a sterile 15 µm sieve, separated from the enzyme solution by centrifugation at 600 x g for 10 min, washed twice, resuspended in 2 ml Phos-KCl, and stored at 4°C.
P. infestans: Protoplasts were isolated as described by Judelson *et al* (1991), with the following modifications. Sterile pea broth extract (300g frozen peas/litre distilled H_2O) was used instead of ALBA medium for liquid culture. The lytic enzyme solution consisted of 5

mg/ml lysing enzymes (as before) in KC osmoticum.

S. nodorum: Protoplasts were isolated as described by Cooley et al (1988) with the exception that sterile liquid complete medium (Newton & Caten 1988) was used instead of CzV8CS medium for liquid culture.

Fluorescein diacetate (FDA, Sigma) was used at 0.01% (w/v) to determine protoplast viability. After incubation for 2 min in the dark, protoplasts were examined using UV microscopy. Respiration rates were determined using an oxygen electrode (Hansatech).

RESULTS AND DISCUSSION

Table 1. Protoplast yield and viability data.

Species	Mean yield of protoplasts /g fresh weight ± SE	Mean consumption O_2 per protoplast nmol/h ± SE
B. cinerea	$1.7 \times 10^7 \pm 7.4 \times 10^5$	$1.7 \times 10^{-4} \pm 8.0 \times 10^{-6}$
P. infestans	$2.0 \times 10^5 \pm 1.5 \times 10^4$	$8.2 \times 10^{-3} \pm 3.9 \times 10^{-3}$
S. nodorum	$3.2 \times 10^7 \pm 2.1 \times 10^6$	$2.7 \times 10^{-5} \pm 4.0 \times 10^{-6}$

Data from 10 replicates revealed high protoplast yields, high physiological intactness and linear rates of respiration (table 1). The use of these systems to study fungicide action is in progress.

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A MONOCLONAL ANTIBODY TECHNIQUE FOR INVESTIGATING PREDATION ON VINE WEEVIL IN SOFT FRUIT PLANTATIONS

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ABSTRACT

In three separate fusions, a panel of monoclonal antibodies (MAbs) was raised against the vine weevil *Otiorhynchus sulcatus* for use in studying predation on the eggs, larvae and adults of this species by polyphagous predators. Some of the MAbs recognised egg or adult antigens, and others two or three developmental stages. The MAbs did not cross-react with predatory arthropod species. This paper describes the development of these monoclonal antibodies and discusses their rôle in evaluating the consumption of vine weevil by native polyphagous predators found in soft fruit plantations.

INTRODUCTION

The vine weevil, *Otiorhynchus sulcatus* is a pest of over 150 ornamental, soft fruit and coniferous plant species throughout temperate Europe and coastal regions of the USA, Canada, Australasia and Japan (Moorhouse *et al.*, 1992). With respect to the soft fruit industry, it is now the major pest on strawberry crops and an increasing problem on blackcurrants causing significant reduction in yields. The main damage is caused by the larvae feeding on the root system, while adults cause cosmetic damage to leaves and contaminate mechanically harvested fruit.

A number of polyphagous predators such as carabid beetles, staphylinids and spiders are found in soft fruit plantations and may feed on vine weevil eggs, larvae or adults. The nocturnal behaviour and small size of these predators make direct observation of feeding difficult. Immunological methods, based on polyclonal antibodies, have been used in the past to analyse predator gut contents for the presence of prey antigens (Greenstone, 1996). This approach to studying predator-prey relationships has now been advanced by the development of monoclonal antibody technology.

MATERIALS AND METHODS

Monoclonal antibodies were produced by three separate fusions, one against each developmental stage (egg, larva and adult), following a protocol modified from Galfre and Milstein (1981). Supernatant screening of positive hybridomas was performed first against homologous and heterologous vine weevil antigens, and then against predator proteins, using an indirect ELISA (Voller *et al.*, 1976). Only cell lines that demonstrated no cross-reactivity with predator species were cloned.

Dry pitfall traps were used in strawberry and blackcurrant crops to determine which predatory species were present, and to collect predators for testing. The traps were emptied

daily and potential vine weevil predators stored at -80 °C to await the completion of testing of the MAbs.

RESULTS AND DISCUSSION

The developmental stages of vine weevil recognised by the 15 MAbs produced are shown in Table 1. Some were specific to egg or adult antigens, and others to two or three stages. In addition to recognising all vine weevil stages, EMA 133 also recognised all predator species, and was therefore developed as a positive control.

Table 1. Specificity of MAbs produced

Monoclonal Antibodies	Stage(s) recognised by the MAbs
EMA 161, EMA 162	E
EMA 130, EMA 131, EMA 135, EMA 151, EMA 152	A
EMA 134, EMA 149, EMA 154	L,A
EMA 159, EMA 160	E,A
EMA 122, EMA 150	E,L,A
EMA 133	E,L,A + all predators

E,L,A = vine weevil eggs, larvae, adults respectively

The use of these MAbs in an indirect ELISA provides a rapid and highly sensitive technique for the screening of field caught predators. Pitfall catches have identified a large and diverse fauna of polyphagous predators in soft fruit plantations. Most common amongst these were *Harpalus rufipes*, *Pterostichus melanarius*, *Pterostichus madidus*, *Harpalus aeneus*, *Nebria brevicollis* and *Calathus fuscipes*. None of these predators cross-reacted with the MAbs other than EMA 133. Following further screening of the MAbs and tests to determine antigen decay rates, these MAbs will be used to identify which species are important consumers of each vine weevil stage.

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A MOLECULAR TECHNIQUE FOR EXAMINING THE GUT CONTENT OF PREDATORY MITES

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ABSTRACT

A PCR based method has been developed to detect prey DNA in the guts of predators. Primers have been developed to amplify DNA from the mite *Orthotydeus caudatus*, which is believed to be prey for the predatory mite, *Typhlodromus pyri*. If specific primers can be developed for a range of potential prey species, many predators collected from the field can be screened, making this a very powerful technique for predator-prey analysis of mites/insects from the field.

INTRODUCTION

Traditional biochemical methods of predator-prey analysis in entomology involve isoenzyme analysis by electrophoresis to detect prey proteins in the gut of a predator (Murray & Solomon 1978). However, in some cases these methods are not appropriate, for example when a reproducible enzyme fingerprint cannot be found. Therefore, a PCR based method has been developed to detect prey DNA in the guts of predators.

RAPD-PCR (Williams *et al.* 1990) has the advantage that no information about the sequence of specific genes is required to obtain DNA markers; RAPD-PCR produces a random sample of the genome similar to the protein fingerprint obtained in isoenzyme electophoresis (applications of RAPD-PCR in entomology reviewed by Loxdale *et al.* 1996).

The aim of this work is to detect prey mite DNA in the gut of a predator which has recently fed, to investigate the feeding relationships among mites in apple orchards. Specific primers have been developed from DNA fragments generated by RAPD-PCR amplification of DNA extracted from the mite *Orthotydeus caudatus*, which is believed to be prey for the predatory mite, *Typhlodromus pyri*.

METHODS

DNA was isolated from mites using Chelex 100, a chelating matrix, as described in Hoy 1994. This method is quick and simple; DNA suitable for PCR can be extracted in 1h.

The extracted DNA from 30 prey mites was used in RAPD-PCR with an annealing temperature of 52°C; 10 mer random primers were used to amplify arbitrary short pieces of DNA flanked by the random primer sequence. A number of DNA fragments were produced for use as genetic markers. These pieces of DNA were cloned into the plasmid vector pUC19, and the bacterium *Escherichia coli* was transformed with this plasmid. Once the mite DNA had been incorporated into the bacterium, it was sequenced. It was then possible to design specific primers for detection of pieces of mite DNA inside predators.

These primers were used in conventional PCR with an annealing temperature of 60°C, to give a species specific band to determine the presence of prey DNA inside the guts of predators.

RESULTS AND DISCUSSION

Several primers for amplifying DNA from *O. caudatus* were designed and tested in PCRs. Other potential prey species present in apple orchards such as *Panonychus ulmi, Aculus schlechtendali, Zetzellia mali*, and mites from the family Tarsonemidae were included in the PCR to check their specificity. Some of the primers amplified several prey mite species. In this instance the primers cannot be used in a one-step PCR to determine the presence or absence of prey mite DNA, but cutting the PCR products with restriction enzymes could reveal different sized DNA products which could then be used to distinguish between prey species.

The most useful primer amplified only DNA from *O. caudatus*. This primer has been able to amplify DNA from DNA extracts which have been diluted to contain as little as 100,000th of a mite. This level of sensitivity is clearly suitable for detecting prey DNA inside the guts of predators. Further research will include the study of decay curves to investigate how long after feeding the prey mite can be detected in the gut of the predator.

CONCLUSION

Once specific primers have been designed from the target prey DNA, many predators collected from the field can be screened in a single day, making this a very powerful technique for predator-prey analysis of mites/insects from the field. If specific primers can be developed for a range of potential prey species, a DNA extract from a predator can be split and used in separate PCRs using different primers. This would enable the detection of all the prey species eaten by the predator, useful for example in larger polyphagous insects that may feed on several prey species in a relatively short time.

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EFFECT OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA CARPOCAPSAE* ON *RADOPHOLUS SIMILIS* INVASION INTO BANANA ROOTS

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ABSTRACT

An application of *S. carpocapsae* around banana roots resulted in a reduced invasion by *R. similis*. An increasing concentration of *S. carpocapsae* applied did not result in an increasing reduction of invasion by *R. similis*. A treatment with sonicated *S. carpocapsae* produced a lower *R. similis* invasion although not significantly so.

INTRODUCTION

Entomopathogenic nematodes of the genus *Steinernema* have become established as biological pesticides and are especially successful for the control of soil insect pests. Although entomopathogenic nematodes pose no environmental risk, their inundative releases to the soil environment will have some sort of environmental impact. The interaction of the introduced nematodes with the existing organisms in the soil ecosystem is still very unclear. In this study the interaction between the entomopathogenic nematode *Steinernema carpocapsae* and the burrowing nematode *Radopholus similis* is examined, concentrating on the invasion ability of *R. similis* into a host plant's roots.

MATERIALS AND METHODS

Three greenhouse experiments using banana (cv. Grande Naine) were carried out. For all experiments *S. carpocapsae* (J3s) and *R. similis* (mixed inoculum) were each applied in a 5 ml water suspension around the base of the plant simultaneously. After a designated period the roots were harvested and the number of *R. similis* inside the roots was estimated. For the first experiment 15 plants received 500 *R. similis*. Two-thirds of the plants received either 5000 or 25000 *S. carpocapsae*. The roots were harvested after 45 days. For the second experiment 25 plants were divided over five treatments all of which received 500 *R. similis* but four also received various concentrations of *S. carpocapsae*: 5000, 25000, 50000 and 100000. The roots were harvested after seven days. For the final experiment 12 plants received 3000 *R. similis*. Four received 25000 *S. carpocapsae* and four received a suspension of 25000 sonicated *S. carpocapsae* (sonication was for 10 min to release the symbiotic bacteria from the gut). The roots were again harvested after seven days.

RESULTS AND DISCUSSION

Results from experiment 1 (Table 1) were inconclusive and showed no significant differences ($p>0.05$) between treatments. *R. similis* that invaded had, after 45 days, gone

through several generations evening out a possible effect at invasion. Experiment 2 (Table 2) was harvested earlier and showed that the presence of *S. carpocapsae* significantly reduced ($p<0.05$) the number of *R. similis* invading the banana roots. The average number of *R. similis* invading the roots of all four *S. carpocapsae* treatments combined was 147 (±55), a 52% reduction compared to the control. Although in the third experiment (Table 3) the numbers of *R. similis* in the 25000 *S. carpocapsae* and in the 25000 sonicated *S. carpocapsae* treated plants were both lower than in the control plants these were not significantly different ($p>0.05$).

Table 1: Experiment 1: Estimated number of *R. similis* per root system after 45 days. Figures are the means (±sd) of 5 replicates per treatment.

Treatment	Root fresh weight (g)	*R. similis*
Control	0.17 ±0.10 a	2630 ±2385 a
5000 *S. carpocapsae*	0.14 ±0.08 a	2581 ±2436 a
25000 *S. carpocapsae*	0.14 ±0.14 a	3046 ±3383 a

Table 2: Experiment 2: Estimated number of *R. similis* per root system after 7 days. Figures are the means (±sd) of 5 replicates per treatment (except for the control where 1 replicate was lost).

Treatment	Root fresh weight (g)	*R. similis*
Control	1.31 ±0.66 a	285 ±51 a
5000 *S. carpocapsae*	1.36 ±0.61 a	170 ±89 b
25000 *S. carpocapsae*	1.44 ±0.64 a	135 ±41 b
50000 *S. carpocapsae*	1.76 ±0.85 a	151 ±54 b
100000 *S. carpocapsae*	1.56 ±0.72 a	132 ±28 b

Table 3: Experiment 3: Estimated number of *R. similis* per root system after 7 days. Figures are the means (±sd) of 4 replicates per treatment.

Treatment	Root fresh weight (g)	*R. similis*
Control	16.51 ±2.68 a	355 ±50 a
25000 *S. carpocapsae*	14.26 ±1.52 a	214 ±108 a
25000 sonicated *S. carpocapsae*	16.06 ±3.29 a	262 ±71 a

Figures followed by the same letter are not significantly different from each other (Student's t-test, $p>0.05$).

A possible explanation why *S. carpocapsae* should affect the invasion ability of *R. similis* is competition for space. If both nematodes are attracted to the root area, *R. similis* would be at a disadvantage in trying to locate and penetrate suitable invasion sites because of the introduction of large numbers of *S. carpocapsae*. Alternatively, the cause might be a nematicidal property of the symbiotic bacteria of the entomopathogenic nematode. However, the sonicated *S. carpocapsae* treatment did not reduce invasion more than the live *S. carpocapsae* treatment. Further experiments are needed to find out why entomopathogenic nematodes affect plant parasitic nematodes' infectivity.

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PERFORMANCE OF ALKYL POLYGLUCOSIDES AS SPRAY DEPOSITION AGENTS

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ABSTRACT

The spray retention-enhancing properties of a series of alkyl polyglucosides (APs) with chain-lengths ranging from C_4 - C_{16} were examined quantitatively using fluorescein as a marker dye. Their relative efficiencies could usually be related to the corresponding dynamic surface tensions of bulk solutions, as well as to their effects on atomization and resultant spray quality.

INTRODUCTION

Surfactants, especially those based on ethylene oxide (EO), are widely used as adjuvants in formulations of crop protection chemicals in order to optimise their delivery, uptake and biological activity when applied to foliage. However, because of increasing concerns about the environmental impact and safety of some ethoxylated surfactants, in particular ones derived from alkylphenol and tallowamine hydrophobes, more benign replacements are currently being sought by the agrochemical industry. APs are a class of nonionic surfactants which apparently do not constitute an environmental risk, even under unrealistic worst-case conditions (Steber *et al.*, 1995) Their potential as spray deposition agents was assessed in the present investigation.

MATERIALS AND METHODS

APs and EO-based surfactants were obtained from commercial sources. The former were designated using the formula RGP, where, R = number of carbon atoms in hydrophobic alkyl moiety, G = hydrophilic sugar moiety, *viz.*, glucose and P = average degree of sugar polymerisation (e.g. octyl glucoside = C8G1.0). A standard track-sprayer procedure using an even-spray nozzle delivering *c.* 200l/ha, was used for all applications. Each spray solution contained sodium fluorescein in distilled water. Retention on plants (five replicates for each treatment) was quantified by spectrofluorimetry and expressed as deposits per unit emission (DUE) (Courshee, 1960). Dynamic surface tension (DST) measurements of aqueous solutions were recorded using the maximum bubble pressure method. Droplet-sizing (volume median diameter [VMD]) was made using a phase-Doppler particle analyser under spraying conditions simulating those used in retention experiments.

RESULTS

Like EO-based surfactants, addition of APs increased the retention of aqueous sprays on difficult-to-wet plant species, such as oats (Table 1), wheat and rape but not on more wettable species, such as field bean and sugar beet. APs generally reduced the DST of the spray solution and the VMD of the spray cloud in a concentration-dependent manner, as occurs with most EO-based surfactants with EO contents > 8 (Table 1). Low DST and VMD are known to be conducive to retention-enhancement (Anderson & Hall, 1989). The most effective APs had mean hydrophobe chain-lengths between *c.*C_8 and C_{12} which formed translucent solutions in water and were adsorbed rapidly at an expanding air-liquid interface at supramicellar concentrations (DST stability). Our studies have demonstrated that APs with an appropriate hydrophobe composition could probably be used to advantage as spray-modifier adjuvants. At optimal alkyl chain-lengths the performance of APs compares favourably with conventional EO surfactants employed for this purpose.

Table 1. Comparison between the spray retention of aqueous solutions of an AP and two conventional surfactants on oats, and the corresponding VMD and DST of the sprays.

Surfactant (g/l)	DUE[a]	VMD (µm)	DST at 5Hz (mN/m)
C8:10G2.1 (0.2)	554	215	66
C8:10G2.1 (1)	1094	193	48
C8:10G2.1 (5)	1214	183	32
Nonylphenol 9EO (1)	934	203	50
Tallowamine 15EO (1)	884	211	59
None	283	214	71

[a] Back-transformed DUE for a 0.05g/l solution of fluorescein.

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FEASIBILITY OF WEED DETECTION WITH OPTICAL REFLECTION MEASUREMENTS

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ABSTRACT

Spectral reflection differences between crops and weeds are investigated to evaluate the possibilities of reflection measurements for crop/weed discrimination on the field. High resolution spectral measurements were performed on young weeds (14 common species), crops (sugarbeet, maize, potato) and soils. Young weeds and young crop differ significantly in their spectra. Statistical methods were used to find the most discriminating wavelength bands. Classification of the spectra in crop, weeds and soil is possible, based on a limited number of narrow wavelength bands (10 nm wide).

INTRODUCTION

Since weeds are not evenly distributed on a field, the optimal treatment of weeds is also non-uniform. The site-specific weed treatment requires an evaluation of the weed distribution on the field, either beforehand or during treatment. Automatic weed detection is a necessity for cost-effective site-specific weed treatment.

Reflection differences between crops and weeds have been reported by Hahn & Muir (1993) and Brown *et al.* (1995). The feasibility of using reflection measurements for crop/weed discrimination is further investigated.

METHODS

High resolution spectra of leaves of young weeds, crops and soils were measured with the Varian Cary 5 computer controlled spectrophotometer in the 200 to 2000 nm wavelength range. Reflectance spectra were measured, using an integrating sphere and a $BaSO_4$-sample as a reference. The resolution is one measurement per nm for the 200-800 nm range and one measurement per 2 nm for the 800-2000nm range. Spectral measurements were performed on leaves of young weeds (*Stellaria media*, *Polygonum persicaria*, *Polygonum convolvulus*, *Mercurialis annua*, *Solanum nigrum*, *Echinochloa crus-galli*, *Chenopodium album*, *Galinsoga parviflora*, *Urtica dioica*, *Cirsium arvense*, *Sonchus oleraceus*, *Anagallis arvensis*, *Glecoma hederacea*, *Plantago major*) and crops (sugarbeet, maize, potato) and on soils. In order to reduce the number of data in further analysis, the spectra were expressed as a mean value per 10 nm. Statistical tests (F-tests) on the spectral data show that young weeds and young crops differ significantly in their spectra. The SAS procedure STEPDISC was used to find the most discriminating wavelength bands (per 10 nm) for distinction between crop, weed and soil. A selection of 2 to 7 wavelength bands was tested for classifying the measured data in the correct category (crop, weed or soil), using the SAS procedure DISCRIM.

Table 1 shows some of the classification results with beet (100 spectra), maize (58 spectra), potato (63 spectra), weeds (328 spectra) and soils (16 spectra).

The discrimination between beet and weeds posed no problems, because beets have a relative low reflection in the 1950 nm region compared to the other plants. The region around 1950 nm is a water absorption band. Discrimination between maize and weeds was more difficult, due to the similarity between maize and cockspur in their spectra. The classification improved by performing it in 2 steps:
1. the discrimination between maize and cockspur (1 category), the other weeds and soil,
2. the discrimination between maize and cockspur.

The discrimination between potato, weeds and soil was quite good with only 3 wavelength bands. Only *Chenopodium album* was sometimes misclassified as a potato.

Table 1. Classification results

categories	wavelength bands (central wavelength in nm)	% incorrect classifications
beet/weed/soil	755, 1925, 1715	0.0 %
beet/weed/soil	755, 905, 1925	0.7 %
maize & cockspur/weed/soil	1285, 455, 355, 685	1.1 %
maize/cockspur	1085, 645, 695	0.0 %
potato/weed/soil	765, 515, 1935	0.5 %
potato/weed/soil	765, 675, 515, 1935	0.2 %

CONCLUSION AND DISCUSSION

Classification of the spectra in crop, weeds and soil is possible, based on 3 to 7 narrow wavelength bands. This is of course a simpler classification than in field circumstances. Reflection measurements on field will contain a component from crop, from weeds and from soil. The measured combination has to be converted to the different composing reflections, allowing for quantification of the surface of bare soil, the surface covered with crop and the surface covered with weeds in the field of view of the sensor. A calibration or continuous correction for the variability in the spectral responce of the soil is needed to get a fair estimation of the quantities of soil, crop and weed in the field of view of a sensor.

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SMALL MAMMAL ACTIVITY IN NEW HEDGEROWS

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ABSTRACT

The creation of new hedgerows offers novel habitats to rodent species which are considered to be agricultural pests. New hedgerows with four different treatments and established hedgerows are considered in terms of small mammal species present. Results suggest use of new hedgerow areas is important with respect to possible pest potential.

INTRODUCTION

Hedgerows are extensions of woodland habitats (Eldridge, 1971). In woodland habitats, there have been numerous studies of small mammals including population studies, effects of food supply on distribution (Flowerdew, 1976) and seasonal fluctuations (Gurnell, 1978). These have demonstrated the importance of food resources in the maintenance of population levels. There are implications for newly established hedgerows as they may attract small mammals looking for resources and act as linear corridors (Zhang and Usher, 1991).

Research into small mammals in agricultural environments has concentrated on direct effects, such as those of pesticides (Greig-Smith *et al.*, 1992) and upon population dynamics and behaviour in established hedgerows and adjacent woodland. The response of small mammals as potential agricultural pests in new hedgerows has not, however, been studied previously. The reported study focusses on the seven month period following plantation of a new Blackthorn (*Prunus spinosa*) hedgerow.

MATERIALS AND METHODS

Two blackthorn hedgerows were planted in late 1994. Each hedgerow was subject to four different treatments (20m x 2m) replicated a total of six times between the two hedgerows. Treatments involved sowing with *Dactylis glomerata* and *Festuca rubra*, untreated, crop or half treatment/half crop. The crop was identical to that adjacent to the hedgerow. In addition three established hedgerows were considered, one on a bank, one adjacent to a road and a third on a farm track. Small mammals were trapped using standard Longworth live trapping techniques. Hedgerows were trapped for five nights in every month. Two traps were placed in the centre of each 20m treatment and at 20m intervals along the established hedgerows.

RESULTS AND DISCUSSION

When species were considered together (Table 1) there were no significant differences between new and established hedgerows ($p=0.071$). In contrast, *Clethrionomys glareolus* is present in much larger numbers in the established hedgerows than in the new ($p<0.001$). This is in agreement with

studies that bank voles are reliant on vegetation structure and density (Boone and Tinklin, 1988). The number of *Microtus agrestis* is significantly higher (p=0.001) in the fully treated plots than anywhere else in the study area. This suggests that vegetation may be important as a source of habitat or as food source for this species.

Table 1. Mammals caught in seven hedgerow types: NFT - new, fully treated; NHT - new, half treated; C - crop; U - untreated; EB - established bank; RH - road hedge; TH - track hedge. Each value is the mean number of individuals caught per trap from seven months trapping data.

	1 (NFT)	2 (NHT)	3 (C)	4 (U)	5 (EB)	6 (RH)	7 (TH)	LSD P=0.05
All species	0.186	0.156	0.18	0.162	0.256	0.171	0.123	0.105
A sylvaticus	0.120	0.183	0.164	0.148	0.189	0.097	0.043	0.115
C. glareolus	0.010	0.002	0.007	0.005	0.051	0.071	0.043	0.038
M. agrestis	0.055	0.012	0.005	0.005	0.013	0.003	0.026	0.037

Numbers of *Apodemus sylvaticus* are shown to be significantly greater in the new hedgerows than in the established areas (p=0.014). This suggests that the new hedgerow is also important as a food source or habitat for this species. This indicates that the wood mouse (*A. sylvaticus*) is not confined to hedgerows and may have a much greater pest potential in areas such as cereal crops than has previously been thought.

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