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(Incorporating the Student Poster Competition)

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Poster Papers: 5I-1 to 5I-15

Growth reduction of cotton (*Gossypium hirsutum*, L.) caused by bermundagrass (*Cynodon dactylon*, L). A case of allelopathy

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ABSTRACT

The influence of *Cynodon dactylon* on cotton growth, was studied in the experimental field of the Agricultural University of Athens, Greece, in 2002. In this experiment, *C. dactylon* rhizomes were planted adjacently to cotton plants in sequential times (0,21,42 days after sowing). At the end of growth period, several characteristics of cotton plants were recorded. Results clearly show that cotton growth was suppressed. Suppression depended on the date of rhizome planting and it was more pronounced in plots where *C. dactylon* was planted at the same time as sowing cotton.

As soil analysis as well as pH evaluation didn't reveal any differences in either soil nutrient status or soil pH, it is concluded that cotton growth reduction can be attributed to allelopathy which is developed by the weed.

INTRODUCTION

Bermundagrass (*Cynodon dactylon* L, Graminae) is a perennial weed. It is one of the most injurious and difficult to control weeds worldwide. *C. dactylon* grows in a wide range of climates (in temperate zones, in Mediterranean region, in sub-tropics, or in the tropics). It is a common weed in cotton fields (Boz, 2000). Due to its dense root system, it is very competitive to crops, depriving them from soil nutrients and water. If bermundagrass control fails, crops are suffering considerable yield losses.

Also in Greece, cotton is suffering yield losses from bermundagrass. Farmers are concerned about *C. dactylon* and not rarely they abandon patches in cotton fields where control of bermundagrass fails. So far, problems in cotton performance were attributed to competition for nutrients and water. Field observations triggered further research to be undertaken in the research site of Plant Production Laboratory in the Agricultural University of Athens. This research effort is targeted in studying in-depth the effect of *C. dactylon* on cotton, by evaluating performance of cotton plants under the influence of the weed.

MATERIALS AND METHODS

In order to study the influence of *C. dactylon* to cotton, a pot experiment was established in May 2002, in the research site of the Agricultural University of Athens.

Cotton seeds (varieties Campo and Millenium), were sown in 17 cm pots, 1-2 cm under the soil surface, which was taken from the topsoil of the experimental field. In each pot, one cotton plant was growing. Total number of plots was 96 (48 for variety Campo and 48 for variety Millenium). In order to influence the growth of cotton plants, *C. dactylon* rhizome cuttings taken from stands in the experimental field, were planted in the pots. Cuttings were 4-6 cm long, containing 3-4 buds. Rhizome cuttings were planted near the pot circumference. Plots were allocated to treatments as follows:

For variety Campo (Treatments A-D): A) 12 pots where rhizomes were planted at the day of sowing (0 DAS), B) 12 pots where rhizomes were planted 21 DAS, C) 12 pots where rhizomes were planted 42 DAS, D) 12 pots where cotton was growing with no added rhizomes.

For variety Millenium (Treatments E-H): E) 12 pots where rhizomes were planted at the day of sowing (0 DAS), F) 12 pots where rhizomes were planted 21 DAS, G) 12 pots where rhizomes were planted 42 DAS, H) 12 pots where cotton was growing with no added rhizomes.

During the experiment all plots remained in the field and were watered regularly (at 1-2 days intervals). Fertilizer was applied three times (Complesal Fluid 12-4-6, enriched with micronutrients, 20 ml/10 litres H_2O). The above ground parts of *C. dactylon* were harvested regularly (at 40-45 days intervals) and preserved into a freezer for subsequent chemical analysis.

At the end of the experiment, cotton plants were harvested and stem height, stem diameter (at the soil line), number of buds on the stem and, root surface were measured. Then, plant parts were oven dried for 3 days at 65 °C and stem dry matter (excluding leaves weight) and root dry matter were recorded.

In order to estimate nutrient levels in the soil, soil samples were taken from plots of each treatment and values of N, P, K, pH were recorded.

RESULTS

At the end of the experiment, cotton plants were harvested. Characteristics measured and values recorded for both varieties, are presented in Table 1.

Table 1. Plant characteristics for cotton plants grown under the influence of *C. dactylon*. Treatments A-D refer to variety Campo and E-H to variety Millenium

Treatments	Stem height (cm)	Stem diameter (cm)	Stem DM (g)	Number of buds	Root DM (g)	Root surface (mm^2)
A	8.42	0.39	0.69	11.9	0.646	687.21
B	8.46	0.40	0.69	14.8	0.679	742.97
C	13.93	0.53	1.56	18.9	1.405	1694.39
D	20.76	0.64	2.55	27	1.939	3788.06
E	9.71	0.36	0.55	12.9	0.368	395.81
F	11.54	0.45	0.88	20.1	0.726	641.7
G	15.73	0.57	1.37	22.2	0.812	841.99
H	24.68	0.68	2.69	28.8	1.609	2032.81

When *C. dactylon* was planted in proximity to cotton seeds (variety Campo) on the day of sowing, stem dry matter was reduced by 72.94%, root dry matter was reduced by 66.68% and root surface was reduced by 81.85%. For the same variety, when *C. dactylon* was planted in proximity to cotton plants 42 days after sowing, stem dry matter was reduced by 38.82%, root dry matter was reduced by 27.53% and root surface was reduced by 55.27% (Table 2).

Table 2. Percentage reduction of plant characteristics compared to untreated cotton plants, when cotton plants (variety Campo) were grown under the influence of *C. dactylon*

Treatments	Stem height	Stem diameter	Stem DM	Number of buds	Root DM	Root surface
A	59.44	39.06	72.94	55.92	66.68	81.85
B	59.24	37.50	72.94	45.18	64.98	80.38
C	32.89	17.18	38.82	30.00	27.53	55.27
D	0	0	0	0	0	0

When *C. dactylon* was planted in proximity to cotton seeds (variety Millenium) on the day of sowing, stem dry matter was reduced by 79.55%, root dry matter was reduced by 77.12% and root surface was reduced by 80.52%. For the same variety when *C. dactylon* was planted in proximity to cotton plants 42 days after sowing, stem dry matter was reduced by 49.07%, root dry matter was reduced by 49.53% and root surface was reduced by 58.57% (Table 3).

Table 3. Percentage reduction of plant characteristics compared to untreated cotton plants, when cotton plants (variety Millenium) were grown under the influence of *C. dactylon*

Treatments	Stem height	Stem diameter	Stem DM	Number of buds	Root DM	Root surface
E	60.65	47.05	79.55	55.20	77.12	80.52
F	53.24	33.82	67.28	30.20	54.87	68.43
G	36.26	16.17	49.07	22.91	49.53	58.57
H	0	0	0	0	0	0

Overall for both varieties, stem dry matter, root dry matter and root surface appear to be the most sensitive characteristics when cotton is influenced in the field from bermundagrass.

DISCUSSION

C. dactylon is receiving growing interest in studying the adverse effects that causes to crops. Gonzales, *et al.* (2002), tested leaf and stem extracts of *C. dactylon* on seeds of oat, wheat, sorghum and bean. In this study, inhibitory effects on seed germination and reduced dry matter accumulation were observed. In another study Delachiave *et al.* (1999), studied the effect of aqueous extracts of *C. dactylon* on the germination of cucumber, corn, bean and tomato seeds. The extracts inhibited tomato and cucumber seed germination but didn't inhibit maize and bean seed germination. Moreover the allelopathic impact of *C. dactylon* on growth and yield of sugar beet was studied by Fayed *et al.* (1999): Aqueous leachates of bermundagrass reduced percentage germination, plumule and radicle length and fresh and dry weight of sugar beet

seedlings, whereas residues of decayed weed roots reduced plant height, number and area of sugar beet leaves, size, fresh and dry weights.
In the experiment we conducted in the research site of the Agricultural University of Athens, cotton growth was negatively influenced by bermundagrass. Both cotton varieties sowed, appeared to be susceptible to weed interference. All characteristics measured were suppressed. Reduction depended on the time of rhizome planting in proximity to cotton plants. Suppression was more severe when rhizomes were planted in proximity to cotton plants on the day of sowing.

In our experiment, another implication of bermundagrass allelopathy to cotton was revealed: Cotton roots not only were thin and undeveloped (which is described by the root dry matter and the root surface measurements), but root hairs were absent (for all treatments that *C. dactylon* was growing in proximity to cotton). This is in accordance with Mez (1961), who found that when *C. dactylon* was growing adjacently to vines, production of root hairs of vines was reduced. Obviously root hairs are necessary for proper nutrition and water uptake from plants. Absence of root hairs results in inferior root functioning and poor crop performance.

Additionally, soil analysis didn't reveal any deficiencies in nutrients in pots where cotton was growing simultaneously with *C. dactylon*, compared to pots where cotton plants were growing solely. This also implies that stunting and plant parts poor development can be attributed not to competition for nutrients but to allelopathy which is exhibited by *C. dactylon*.

Further research is continued in the Agricultural University of Athens to study performance of cotton plants when they interfere with *C. dactylon* and to identify chemical compounds the weed produces in order to affect cotton plants.

ACKNOWLEDGEMENT

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Compatiblity of the candidate bioherbicide *Microsphaeropsis amaranthi* with chemical herbicides and adjuvants in tank mixture

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ABSTRACT

Waterhemp, *Amaranthus tuberculatus* Sauer., is a serious weed of cropping systems in the Midwestern United States. *A. tuberculatus* exhibits resistance to a wide range of herbicide chemistries and is a frequent escape from existing management systems. We are investigating the potential of the plant pathogenic fungus, *Microsphaeropsis amaranthi*, to provide supplemental control of *A. tuberculatus*. Applications of sub-lethal rates of glyphosate predisposed *A. tuberculatus* to attack by *M. amaranthi*. Tank mix experiments designed to simulate mixture in a spray tank during field applications measured the ability of conidia to germinate after exposure to selected herbicides and adjuvants. These experiments reveal the potential for positive interactions between herbicides and *M. amaranthi*, but underscore the practical difficulties of developing integrated systems that exploit these interactions in the field.

INTRODUCTION

Microsphaeropsis amaranthi is a plant pathogenic fungus, host specific to the genus *Amaranthus* (Mintz *et al.*, 1992). Waterhemp, *Amaranthus tuberculatus*, is a major weed of Midwestern cropping systems, and control is difficult due, in part, to populations that are resistant to s-triazines, diphenylethers, ALS inhibitors and glyphosate (Patzoldt *et al.*, 2002; Zelaya & Owen, 2002). Furthermore, *A. tuberculatus* tends to be a late emerger and germinates over a period of several months which puts higher demands on weed management programs (Hartzler *et al.*, 1999).

A common weakness of bioherbicides is a high level of host specificity. This reduces their utility in multi-species weed communities, which are the norm. In order to be of significant value as a bioherbicide for the control of waterhemp, *M. amaranthi* would need to be compatible with chemical herbicides used for the control of other weed species. It would be particularly desirable for *M. amaranthi* to be tank mixed with chemical herbicides and co-applied. The objectives of this research were to investigate the ability of glyphosate to predispose *A. tuberculatus* to infection by *M. amaranthi*, and to evaluate the compatibility of *M. amaranthi* with chemical herbicides in tank mixture.

MATERIALS AND METHODS

Split application experiment

A. tuberculatus was pre-treated overnight in a 0.005% (w/v) solution of Gibberellic acid (GA_3) and then sown into small (36x36 mm) plastic modules containing potting mix (MetroMix 360,

Scott's Co., Marysville, Ohio). Plants were sprayed (225 litres/ha, XR8003 tip, 240 kPa) at the 3-4 leaf stage with various rates of glyphosate (0, 0.125, 0.25, 0.5, and 1X, where 1X=0.63 kg a.e./ha). Conidia of *M. amaranthi* were applied 24 h later at $3x10^6$ conidia/ml in the same way. Plants were placed in a dew chamber for 12 h at 18°C and then returned to the greenhouse for 10 d, harvested and dry weights measured.

Tank mix experiments

Tank-mix experiments were developed to simulate the impact of herbicides and adjuvants upon *M. amaranthi* in the spray tank environment. Conidia of *M. amaranthi* were harvested from cultures on V8 juice agar plates and strained through three layers of cheesecloth. Concentrations were adjusted to $1.5x10^6$ conidia/ml using a hemacytometer, and incubated with herbicide/adjuvant solutions in cell plates (15 mm) on an orbital shaker for 2 h at concentrations of 0, 0.25X, 0.5X, 1X, 2X, and 4X (X=recommended label rate). Conidial germination was stimulated by the addition of 1 ml of ½ strength potato dextrose broth and germination counted under a compound microscope using 4 views of 25 conidia for each of 4 replicates after a further 3 h incubation. Herbicides/adjuvants tested were: Gramoxone Max (paraquat), Beacon (primisulfuron methyl), FirstRate (cloransulam-methyl), Pursuit (imazethapyr), Aim (carfentrazone-ethyl), Sencor (metribuzin), Ultra Blazer (acifluorfen), Cobra (lactofen), Aatrex 4L (atrazine), Buctril (bromoxynil), Liberty (glufosinate), Roundup WeatherMax, Roundup UltraMax, Roundup Original, Roundup Custom, Glyphomax Plus, Touchdown, tech. grade K and IPA salts (glyphosate), formulation blank of Glyphomax Plus, Herbimax (crop oil concentrate/COC), Activator 90 (non-ionic), Triton X-100 (non-ionic), Ammonium sulfate (AMS), AG 6206 (alkylpolyglucoside/APG), Tween 20 (non-ionic), Silwett L-77 (organosilicone), Toximol TA15 and TA5 (tallow amines).

RESULTS

Split application experiment

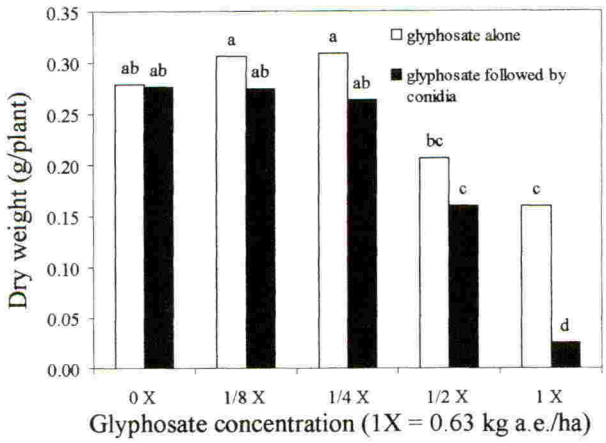

Figure 1. Dry weight of *A. tuberculatus* treated with glyphosate and *Microsphaeropsis amaranthi* in a split-application. Bars with the same letter(s) are not significantly different (P=0.05)

Dry weight reductions of *A. tuberculatus* were caused by glyphosate concentrations of 0.32 kg a.e./ha or greater. Applications of 0.63 kg a.e./ha glyphosate and $3x10^6$ conidia/ml of *M. amaranthi* caused significantly greater dry weight reductions in combination than either did alone (Figure 1).

Tank mix experiments

Of the herbicides tested, Aim and Pursuit caused minimal decreases in germination of *M. amaranthi* conidia, while most herbicides caused a larger decrease: Gramoxone Max, Beacon, FirstRate, Sencor, Ultra Blazer (Figure 2A,C). Some herbicides completely prevented germination at all concentrations tested: Cobra, Aatrex 4L, Liberty, and Buctril. Of the adjuvants tested, COC, AMS, APG, Tween 20, and Silwett L-77 caused a decrease in germinability (Figure 2D). Some adjuvants completely abolished germination at all

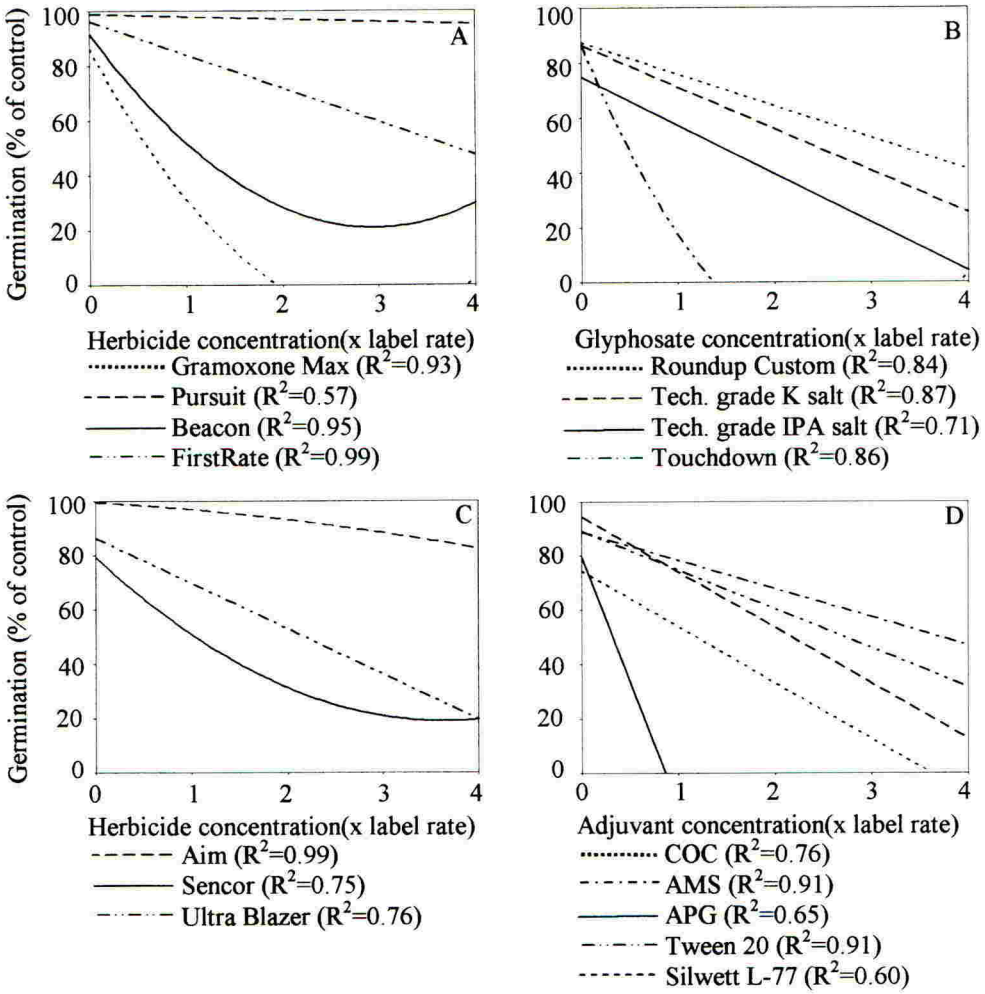

Figure 2. The effects of various herbicides and adjuvants on the germinability of *Microsphaeropsis amaranthi*.

concentrations tested: Activator 90, Triton X-100, Toximol TA15 and TA5. Most glyphosate formulations completely abolished germination at all concentrations tested: Glyphomax Plus, Roundup WeatherMax, Roundup UltraMax, Roundup Original, and a formulation blank of Glyphomax Plus. Glyphosate products lacking in tallow amine surfactants resulted in smaller germination reductions: Roundup Custom, Touchdown, Technical grade K salt, Technical grade IPA salt of glyphosate caused smaller germination reductions (Figure 2B).

DISCUSSION

Application of glyphosate predisposed *A. tuberculatus* to infection by *M. amaranthi* applied 24 h later (Figure 1). This is consistent with the findings of Sharon *et al.* (1992). We hypothesize that this predisposition is due, at least in part, to a reduced phytoalexin defense reaction in *A. tuberculatus* as a result of depleted phenylalanine production from the shikimate acid pathway, the target of glyphosate (Sharon *et al.*, 1992). This interaction suggests that there is potential to exploit *M. amaranthi* for the control of *A. tuberculatus* escapes, particularly in cropping systems using glyphosate resistant crops. When conidia of *M. amaranthi* were incubated in herbicide or adjuvant solutions, in order to simulate the environment of a spray tank during application, germination was inhibited in most cases (Figure 2). In the case of glyphosate products, it was primarily the surfactants employed, most importantly the tallow amine surfactants, that were the primary cause of this impact (Figure 2C, D). We conclude that whilst a physiological interaction exists between glyphosate and *M. amaranthi*, the exploitation of this interaction, and interactions with other herbicides and adjuvants, in the field will be difficult. Future studies will investigate formulations that support the activity of chemical herbicides as well as the germinability of conidia of *M. amaranthi*.

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Structure-activity relationships of herbicidal 3-phenyl substituted derivatives of 1,2,3-benzotriazin-4-ones, 4(3*H*)-quinazolinones, and 2,4(1*H*,3*H*)-quinazolinediones

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ABSTRACT

Structure-herbicidal activity relationships of sixty title compounds were studied. Substitution of a hydrogen on 2, 5, 7, or 8 position on the heterocycle decreased herbicidal activity, and a methyl on 1 position of the 2,4(1*H*,3*H*)-quinazolinediones provided greatest activity. The most active compound was 3-[(2-F-4-Cl-5-propargyloxy)phenyl]-1,2,3-benzotriazin-4-one.

INTRODUCTION

The protoporphyrinogen-IX oxidase inhibitors are structurally very diverse, ranging from diphenylethers to *N*-substituted phenyl heterocyclic carboxamides. High resistance to soil leaching, low toxicity to birds, fish, and mammals, and slow development of weed resistance have made the carboxamides a class of important herbicides.

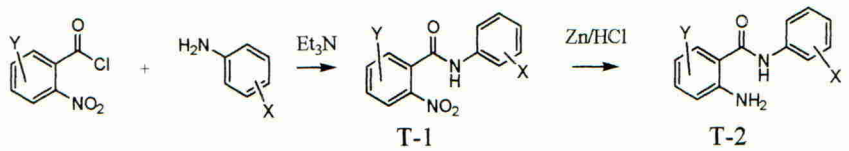
Some 3-monosubstituted phenyl of the 1,2,3-benzotriazin-4-ones (Satzinger & Herrmann, 1972), 4 (3*H*)-quinazolinones (Janiak, 1969), and 2, 4(1*H*,3*H*)-quinazolinediones (Giral, 1978) were found in the 1970s to have fungicidal and pharmaceutical activity. Broadleaf weed control activity was discovered in some analogues of these compounds (Hsu & Li, 2002; Li & Hsu, 2002a, b). Since 1980, a number of groups have carried out structure-activity relationship (SAR) studies on the herbicidal heterocyclic carboxamides (Fujita & Nakayama, 1999). However, no published reports could be found involving SAR studies with the 1,2,3-benzotriazin-4-one, 4(3*H*)-quinazolinone, and 2,4(1*H*,3*H*)-quinazolinedione heterocycles. In this study, we examine 60 analogues from these three heterocycles to investigate the effects on herbicidal activity of substitution that might be helpful in further lead optimization.

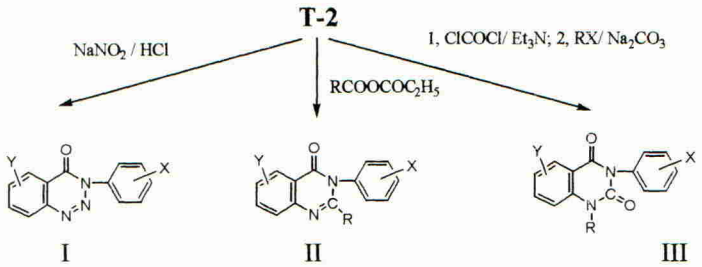
MATERIALS AND METHODS

Chemicals

Compounds were prepared as described in the patents (Hsu & Li, 2002; Li & Hsu, 2002a, b). The structures were confirmed by ^{1}H NMR (Mercury-300). All target compounds were purified with silica gel column chromatography. The eluent was a mixture of ethyl acetate and hexane at various ratios. Final purity of the target compounds was over 90%, as estimated from ^{1}H NMR results. Melting points were determined in capillary tubes and uncorrected.

The synthetic route was as follows:





Herbicidal activity evaluation

Three broadleaf plant species: marigold (*Tagetes spp*), tomato (*Lycopersicon esculentus*), and velvetleaf (*Abutilon theophrasti*) were used for the test. The seeds were allowed to germinate and grow for 14 days. Test plants were selected for uniformity, size and stage of development and then treated with the test compound, returned to the greenhouse and watered. The plants not treated with the compound under evaluation were used as a comparison. The compound to be evaluated was dissolved in acetone and sprayed using a carrier volume equivalent to 187 liters per hectare at 1200g/ha. Two weeks after application of the test compounds, the state of the plants was observed. Each species was evaluated on a scale of 0-100 in which 0 equals no activity and 100 equals total control. The average control of the three broad leaf plant species (represented by AD) was calculated.

RESULTS AND DISCUSSION

Synthesized compounds, along with melting point and herbicidal activity, are presented in Tables 1 to 3. Structure and herbicidal activity relationships of the compounds can be summarized as follows:

1. For 1,2,3-benzotriazin-4-one compounds (series I), when 3-phenyl was monosubstituted, the most active substituent was 4-Cl, while substitution at the meta or ortho position always decreased herbicidal activity (except with 2-F). These effects were also generally true for some additional 4(3*H*)-quinazolinones and the 2,4(1*H*,3*H*)-quinazolinediones not presented in this paper.
2. When 3-phenyl was disubstituted, the combination of 2-F and 4-Cl improved the herbicidal activity.

3. When 3-phenyl was trisubstituted, substituents placed at the 2, 4, and 5 positions generally provided greatest activity. The most active molecule had a F at the 2, a Cl at the 4, and a propargyloxy at the 5 position.
4. For the compounds of series II, introduction of an R substituent on the heterocycle decreased herbicidal activity, whether R was an electron donating or withdrawing group.
5. For the compounds of series III, replacing NH with NCH_3 on the heterocycle always increased herbicidal activity, which could indicate that position 1 of the heterocycle is an H-bond acceptor for binding with the protoporphyrinogen-IX oxidase.
6. Introduction of substituent Y on the heterocycle usually decreased herbicidal activity.
7. Among the three structural moieties represented by series I, II, and III, the 3-phenyl-1,2,3-benzotriazin-4-ones provided greatest weed control activity.

Table 1. Herbicidal activity of 3-phenyl-1,2,3-benzotriazin-4-ones (I)

Compound	X	Y	m.p. °C	AD
I-1	2-Br	H	105-106	0
I-2	2-CF_3	H	143-144	0
I-3	2-Cl	H	116-117	0
I-4	2-CN	H	158-159	0
I-5	2-CH_3	H	163-164	0
I-6	2-CH_3O	H	150-151	0
I-7	3-Br	H	156-157	0
I-8	3-CF_3	H	134-135	0
I-9	3-Cl	H	140-141	0
I-10	3-CN	H	192-193	0
I-11	3-F	H	133-134	0
I-12	3-I	H	143-144	0
I-13	3-CH_3	H	148-149	0
I-14	3-CH_3O	H	123-124	0
I-15	4-CH_3O	H	155-156	0
I-16	4-Br	H	194-195	0
I-17	4-CF_3	H	231-232	0
I-18	4-I	H	134-135	0
I-19	2-I	H	148-149	10
I-20	H	H	149-150	13
I-21	4-CN	H	226-227	23
I-22	2-F	H	140-141	25
I-23	4-CH_3	H	138-139	26
I-24	4-F	H	147-148	37
I-25	4-Cl	H	183-184	46
I-26	2,4 -$(CH_3)_2$	H	126-127	0
I-27	3,4-Cl_2	H	210-211	0
I-28	3,5-Cl_2	H	219-220	0
I-29	2-Cl, 4-F	H	174-175	26
I-30	2,4-Cl_2	H	125-126	30
I-31	2-F, 4-Cl	H	144-145	65
I-32	2,4,6-Br_3	H	128-129	0
I-33	2,4,6-Cl_3	H	126-127	0
I-34	2-F, 4-Cl, 5-C_2H_5OCOO	H	128-129	33
I-35	2-F, 4-Cl, 5-propargyloxy	5-CH_3	oil	40
I-36	2-F, 4-Cl, 5-cyclopentyloxy	H	133-134	41
I-37	2-F, 4-CN, 5-F	H	203-205	53
I-38	2,4,6-F_3	H	173-174	58
I-39	2-F, 4-Cl, 5-$(CH_3)_2CHOCO$	H	183-184	66
I-40	2-F, 4-Cl, 5-propargyloxy	8-Cl	158-160	66
I-41	2-F, 4-Cl, 5-$C_2H_5OCOCH_2O$	H	118-120	88
I-42	2-F, 4-Cl, 5-propargyloxy	7-CF_3	124-125	91
I-43	2-F, 4-Cl, 5-propargyloxy	H	185-186	100

Table 2. The herbicidal activity of 3-phenyl-4-(3*H*)-quinazolinones (II)

Compound	X	Y	R	m.p.°C	AD
II-1	2-F, 4-Cl	H	CF_3	107-109	0
II-2	2-F, 4-Cl	H	CH_3	132-135	30

II-3	2-F, 4-Cl	H	H	207-209	46
II-4	2-F, 4-Cl, 5-propargyloxy	H	CH_3	129-131	36
II-5	2-F, 4-Cl, 5-propargyloxy	8-Cl	H	203-205	43
II-6	2-F, 4-Cl, 5-propargyloxy	7-Cl	H	>250	66
II-7	2-F, 4-Cl, 5-propargyloxy	H	H	206-207	83

Table 3. Herbicidal activity of 3-phenyl-2,4(1*H*,3*H*)-quinazolinediones (III)

Compound	X	Y	R	m.p.°C	AD
III-1	2-F, 4-Cl	H	$C_2H_5CO_2CH_2$	oil	0
III-2	2-F, 4-Cl	H	H	230-232	16
III-3	2-F, 4-Cl	H	$C_2H_5CO_2$	oil	20
III-4	2-F, 4-Cl	H	CH_3CH_2	164-166	30
III-5	2-F, 4-Cl	H	propargyl	224-225	36
III-6	2-F, 4-Cl	H	CH_3	181-182	40
III-7	2-F, 4-Cl	H	$C_2H_5OCH_2$	103-105	41
III-8	2-F-4-Cl, 5-$C_2H_5OCOCH_2O$	H	CH_3	68-71	6
III-9	2-F, 4-Cl, 5-$(CH_3)_2CHOCO$	H	CH_3	70-73	53
III-10	2-F, 4-Cl, 5-propargyloxy	H	CH_3	175-177	66

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Efficacy of plant protection products containing the botanical antifeedant, azadirachtin, against the large pine weevil *(Hylobius abietis)*

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ABSTRACT

Two individual field trials in central and southern Scotland were used to test the efficacy of four different formulations of neem tree *(Azadirachta indica)* seed extract, against the forest pest the large pine weevil, *Hylobius abietis*. Weekly assessments over 8 weeks were made for one field trial which showed that feeding damage by the pine weevil can be reduced by applying neat formulations containing 5% and 1% AZA onto the seedling. Concentrations lower than these did not provide sufficient protection. Using an azadirachtin formulation known to significantly reduce damage, large scale field trials were used to assess damage at the end of the feeding peak for five different dilutions. All dilutions were found to reduce feeding damage significantly. Both trials were tested against the currently used synthetic chemical permethrin.

INTRODUCTION

The large pine weevil (*Hylobius abietis*) is the principle noxious pest in reforestation areas throughout the U.K. and Scandinavia (Bratt *et al*., 2001). Attracted to their breeding grounds by volatile chemicals released from recently felled trees, adult *H. abietis* invade restock sites and lay their eggs in and around tree stumps (Nordenhem *et al.*, 1994). The young stages of *H. abietis* below the ground cause no economic damage, however the adults above the ground feed on the stems of the young seedlings of the new planted crop. Feeding by *H. abietis* scars the phloem of the seedlings, and girdling of the stem base rapidly kills them, non lethal injury can result in reduced seedling growth (Orlander *et al.*, 1999). With populations recorded of up to 250,000 insects per hectare, losses of seedlings can range from 30-100% for up to five years after clearfelling in sites where no plant protection is given. *H. abietis* damage generally occurs when the adults are active between March and October. Although it is generally recognised that there are two feeding peaks for the weevil, feeding is often difficult to predict. As a consequence prophylactic protection is routinely given using the synthetic chemical permethrin to reduce damage. However, the use of permethrin in European forestry has been withdrawn and remaining stocks have to be used up by the end of 2003. Research in recent years has focused on alternative ways to reduce damage, such as mechanical protection, baited traps, cultural techniques and biological control (Eidmann *et al*., 1996; Brixey 1997; Orlander *et al* 1999; Bratt, *et al*., 2001). Organic chemical plant protection has also been considered (Klepzig *et al.*, 1999) including the use of extracts from the neem tree (Beitzen-Heineke *et al.*, 1994; Thacker *et al.*, 2003). This paper assesses the efficacy of four different formulations of neem seed extract as a plant protection product against the feeding activity of *H.abietis*.

MATERIALS AND METHODS

The data were collected from planted trials established in recently cut clear-fell sites in central (Aberfoyle) and southern Scotland (Dumfries). The seedlings used were 3yrs old, 30-40cm height, Sitka spruce *(Picea sitchensis)* seed origin Queen Charlotte Islands, Washington. Trials were planted during the months of April and May. Standard planting methods were employed; the first trial site had been scarified, whereas the other site was not. Planting was done in a randomised block design. The first trial compared the efficacy of three azadirachtin formulations, applied with no dilution; Nivaar® (0.15% AZA), NeemAzal-T/S® (1%AZA) and NeemAzal Paint (5%AZA). Two controls were used, one left untreated and the other treated with permethrin (Permit®). All azadirachtin treatments were applied using a paintbrush to the first fifteen centimetres up from the root collar, at 5ml per tree before planting. The permethrin treatment was applied using the electrodyn sprayer system, also before planting. In total 300 trees were used, with 12 plants per plot and 5 replicates per treatment. The second trial was used to assess the dose response of Bugban® (10%AZA). There were seven treatments and dilutions were made with vegetable oil. Treatments; A- 1000g/l (no dilution), B- 800g/l, C- 600g/l, D- 400g/l, E- 200g/l, F- permethrin & G- vegetable oil. Due to the consistency of treatments A & B application had to be applied by paint pad before planting (10ml dose). The remaining treatments were applied post planting using a forestry spot gun fitted with a narrow cone nozzle and calibrated at 10ml dose per tree. In total 1260 trees were used, with 36 plants per plot and 5 replicates per treatment.

Assessment measured the percentage of bark removed around the seedling stem at 0-15cm from the ground. Trial one was recorded weekly, trial two was recorded once at the end of the first feeding peak (first week in July). The data were analysed using arcsine-squareroot transformation, from which the mean and 95% confidence limits were derived.

RESULTS

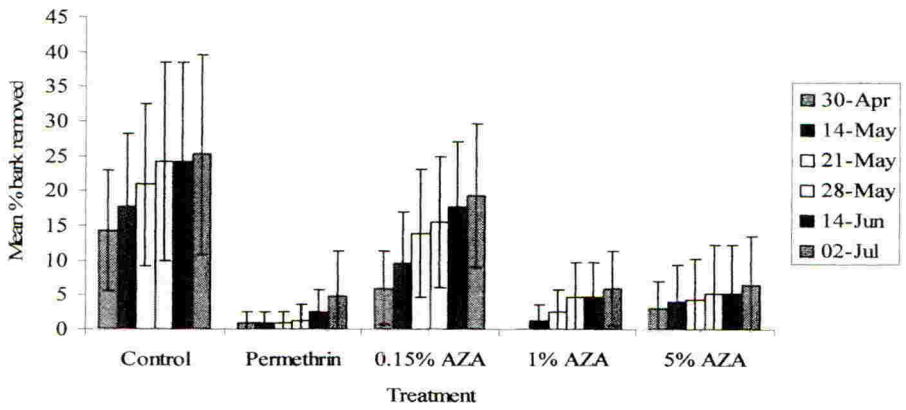

Figure 1. Transformed data, showing mean percentage bark removed 0-15cm above the root collar ±95% confidence limits through time with respect to neem extract formulation.

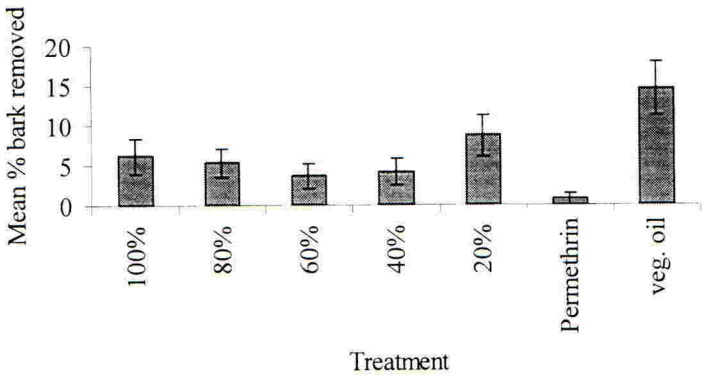

Figure 2. Transformed data, mean percentage bark removed 0-15cm above the root collar ±95% confidence limits with respect to neem extract (10%AZA) concentration.

Figure 1 indicates that for all treatments the percentage bark removed increased over time. In comparison to the control trees the permethrin and azadirachtin formulation (1%) were able to reduce the percentage bark removed significantly. The azadirachtin formulation (5%) reduced feeding damage but not significantly compared to the control. The formulation (0.15%AZA) although reducing damage slightly did not reduce damage significantly and was extremely phytotoxic. Figure 2 using formulation (10%AZA) was able to significantly reduce bark removed for each dilution, though no concentration was as effective as the permethrin treatment.

DISCUSSION

The results indicate that azadirachtin formulations are able to reduce feeding damage by *H.abietis*, though the extent of protection appears to a certain degree to be determined by the concentration. Efficacy of azadirachtin has been correlated with the concentration (Schmutterer 1990), however it would then be expected that the formulation containing 5%AZA would reduce damage more effectively than the 1%AZA formulation (Figure.2), which was not seen. Due to the consistency of the 5% AZA it maybe that it was not as effective as the 1% AZA formulation due to difficulty in applying it resulting in uneven application. The dose response is seen for treatments C(60%), D(40%) & E(20%) in Figure 2, but the higher concentrations A(100%) & B(80%) do not fit in with this trend, which again may be due to application (treatments C, D & E were applied through a calibrated spray system). The formulation with 10%AZA suppressed feeding to a dilution of 20% (2%AZA) and formulation with 1%AZA can also suppress feeding, however this effect appears to be limited in concentrations any lower than this. The formulation with the lowest concentration (0.15%) did not provide significant plant protection and was extremely phytotoxic, resulting in seedling death when applied without dilution. As the other formulations containing higher concentrations of azadirachtin showed no obvious sign of phytotoxicity it is assumed that the toxic effects observed with the low concentration formulation is due to other ingredients within the formulation.

H. abietis has two feeding peaks which tend to occur at the start and end of the summer, with the second feeding peak being more intense than the first. Both Figure 1 & 2 show that the trials received feeding damage but damage to control trees indicate that infestation was not that high. Assessments will continue until after the second feeding peak to gain a clearer picture of efficacy. Further research is required to obtain the optimum concentration and formulation which could provide effective protection of forest transplants against the feeding damage of *H. abietis*.. Although damage may occur to a seedling it is only once the stem has been girdled that the tree will die, the data gathered for this paper only indicated percentage damage, the end of season recording will also consider the survival chances of the seedlings.

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Northern bobwhite chick-arthropod food abundance in insect resistant GM cotton crops

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ABSTRACT

The requirement of insecticide applications to control lepidopteran insect pests in cotton crops may be reduced through the growing of insect resistant genetically modified (IRGM) varieties. A reduction in insecticide applications may benefit some farmland birds. In 2002 and 2003 the feeding ecology of northern bobwhite (*Colinus virginianus*) chicks was investigated in relation to the growing of IRGM and non-IRGM cotton varieties on a farm in central Georgia, USA. Data were collected on habitat use, diet and survival of bobwhite chicks along with chick-arthropod food abundance in the different cotton crops. The methodologies and preliminary results of this study are outlined here.

INTRODUCTION

Although there is currently much debate over the effects GM crops may have on the environment, farmers in the USA have rapidly integrated this new technology into their farming systems. The increase in the percentage of cotton acres planted to GM varieties has been particularly marked. In the state of Georgia, GM cotton varieties accounted for 85% of the total cotton acreage planted in 2001 (USDA statistics, available at http//:www.nass.usda.gov). GM cotton varieties currently available to US farmers are either herbicide tolerant (HTGM), insect resistant (IRGM) or both (Stacked). IRGM cotton varieties introduced in 1996, which accounted for 42% (including stacked varieties) of the cotton acreage in Georgia in 2001, express a gene derived from the bacterium *Bacillus thuringiensis* (*Bt*). Cotton plants expressing this gene produce endotoxins which are toxic to lepidopteran insect pests such as the cotton bollworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, which feed on them. To control these pests in conventional cotton variety crops, multiple applications of insecticide are often used during the growing season (Benedict & Altman, 2001).

Pesticide use, which dramatically increased in the USA over the second half of the twentieth century (USDA statistics) reduces field-arthropod abundance both directly (insecticides) and indirectly (herbicides) (Sotherton *et al.*, 1993). A reduction in arthropod-rich foraging habitats, as a consequence of agricultural intensification, has been cited as a contributory factor for the rapid decline of the northern bobwhite (*Colinus virginianus*) and other farmland bird species in the southeastern USA over the last three decades (Murphy, 2003).

By possibly providing the means for reducing the number of insecticide applications to cotton crops, we hypothesize that IRGM cotton crops may be of greater value as foraging habitats for

northern bobwhite chicks than non-IRGM cotton crops. To test this, a two-year study was initiated in 2002 to investigate the abundance of arthropod chick-food in IRGM and non-IRGM cotton crops.

MATERIALS AND METHODS

The study was conducted on a 300 ha farm in central Georgia, USA. Although cotton is the main crop on the farm, some fields are planted to soya beans, maize, or peanuts. HTGM cotton varieties have been grown on the farm since 1996. Hedgerows, hardwood drains, planted pine stands and scrubland are interspersed amongst cropped fields.

Using a randomized block design, with each field representing an individual block, cotton fields are split in two, with one half planted with a herbicide-tolerant cotton variety and the other with a stacked cotton variety. Fields used in the study ranged in size from 5 to 18.5 ha. Treatments were randomly assigned to field halves. Six and fifteen fields were sampled in 2002 and 2003 respectively.

In July, August and September, vegetation measurements were taken in each study field to determine species composition, structure and cover using methodologies similar to those described by Basore *et al.* (1986). To determine insect abundance, insect samples were collected using a D-Vac suction sampler during the same months. Vegetation and insect samples were taken at 3 random sites at the edge of each plot (< 12m from the plot edge) and 3 random sites in the middle of each plot (>12m from the plot edge).

To investigate brood use of the different cotton varieties, during spring 2002 and 2003, 47 and 41 adult bobwhite quail respectively were caught within 1km of a trial cotton field and fitted with radio-transmitters. During the reproductive season, approximately late May through the middle of September, three diurnal brood locations were taken daily using radio-telemetry until the chicks were 14 days old.

To determine chick-arthropod diet in relation to chick use of the different cotton varieties, chick faeces were collected from brood-roost sites. Ingested arthropods were identified in the faecal samples according to Moreby (1988). At approximately 10 days of age, broods were captured and weighed in order to determine chick daily weight gain (Hammond 2001) and chick survival in relation to diet.

RESULTS

Pest pressure on the cotton crops was low in 2002, possibly due to the drought conditions experienced in the southeastern USA. One application of insecticide was applied to a non-IRGM cotton crop, where the threshold level for the control of bollworms was exceeded.
Although broods utilized habitats adjacent to cotton trial fields no broods were located in these fields in 2002. Of the 7 broods that survived until 14 days of age, the mean Minimum Convex Polygon home range size was 14.73 ha ± 3.63 and mean brood size at hatching was 11.71 ± 0.97. The mean daily weight gain of chicks in five broods that were successfully captured was 0.76g ± 0.08.

Analysis of faecal samples from 8 broods revealed that coleopteran species formed over one-third of the arthropod diet of bobwhite chicks (Table 1). Collectively, Coleoptera, Hymenoptera and Hemiptera made up almost 70% of the chick-arthropod diet.

Table 1. Mean proportions of arthropod orders, calculated from minimum numbers of individuals in faecal samples, in the diet of northern bobwhite broods ($n = 8$) foraging on farmland in central Georgia, USA during summer 2002.

Arthropod Order	Proportion of diet (%)
Araneae	2.3
Coleoptera	36.6
Hemiptera	16.2
Homoptera	11.9
Hymenoptera	16.0
Lepidoptera Larvae	6.0
Orthoptera	9.0
Others	2.0

To date, insect and vegetation samples collected in 2002 have not been analysed. Once analysed, these data in conjunction with the chick dietary data will allow inferences to be made on the value of IRGM cotton for foraging bobwhite broods.

DISCUSSION

In the past few years the possible effects of GM crops on the food resources of farmland birds have received increased scientific attention, particularly in Great Britain (Firbank *et al.*, 2003). The Farm-Scale Evaluations (FSE) project, a three year study that has just been completed in Great Britain, investigated the impacts of HTGM crops on weed seed banks and arthropod-abundance in cropped fields (Firbank *et al.*, 2003). Although these data will allow inferences to be made on the indirect effects of HTGM crops on various farmland bird species, it cannot be used to predict what impacts, positive or negative, will arise from the growing of other GM crops with differing traits i.e. IRGM (Firbank *et al.*, 2003). Therefore, research into the effects of IRGM crops on farmland wildlife is important.

Although there is evidence to suggest that IRGM cotton does reduce the dependency of cotton on insecticide applications (Carpenter *et al.*, 2001), this technology should not be seen as a 'quick fix' solution to increasing the value of cotton fields for farmland wildlife. Instead, IRGM crops should be viewed as an integral part of an IPM system that also incorporates the use of beneficial insects and selective pesticides. Without such management, farmers may forego possible benefits of growing IRGM cotton by applying non-selective insecticides to control other insect pests. Farmer education is therefore crucial to maximising the potential environmental benefit of IRGM crops.

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The survival of Chinese pesticide companies in a global marketplace – an international marketing perspective

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ABSTRACT

It has been acknowledged that the advantageous position of Chinese pesticide companies in world markets is mostly due to their low cost advantage. However, the economic growth in China, increasing price of raw material and real wage based labor certainly will not allow industry to sustain this low cost advantage all the time. In addition to low price, appropriate international marketing strategy must be developed and adopted.

INTRODUCTION

Several multinational basic producers from the United States and Western Europe dominate the world pesticide industry, they account for nearly one-half of the total output. Additionally, hundreds of other smaller firms make selected active ingredients and thousands of formulators offer a variety of different products at retail. At the very least, technological advances offer market niche opportunities, but when patent protection expires, generic competition arises. Strategic alliances in terms of joint venture, merger or acquisition seem a very feasible solution, and prevailing direction since 1990's (Phillips & McDougall, 2001). Agriculture is the fundamental base of the state economy in China, and pesticide, as one of the agricultural production materials, has been playing a very strategic role in this development. So far, the Chinese pesticide industry has become an entire system integrated with research & development, manufacturing and formulation facilities (Hamburger, 2000). While entering into the international market in competition with other rivals from rest of the world, Chinese firms are facing various challenges, such as threats from multinational corporations, technical barriers (registration) and low cost sustainability.

METHODOLOGY

As primary research, quantitative research has been carried out through a survey with postal questionnaires for participants from Chinese trade corporations, basic manufacturers and overseas companies from the countries in the Middle East, South-East Asia, South America, Europe and Africa including importers, and agent/ distributors. Structured telephone interviews were used to carry out qualitative research on selected topics for more comprehensive information. Table 1 shows the number of companies that were asked to participate and the number of respondents categorized by profession. Secondary research has focused on examining the literature covering the areas of international environment, market research, entry modes, international marketing programme and strategic management in international marketing (Albaum *et a.l*, 2002; Bennett, 1995).

Table 1. Survey participants

Category	No. of companies 'being asked'	No. of companies participated	Response rate (%)
Chinese trade corporations	11	7	64
Chinese manufacturers	18	10	56
Overseas companies	20	15	75

RESULTS

Table 2 shows the results from the quantitative survey in order to identify trends & patterns.

Table 2. Survey results

Distribution channel		Communication		Market research		Entry modes	
Through importer	88%	Personal selling	82%	Yes	71%	Direct export	88%
Through local distributor	71%	Exhibition	65%	Some times	18%	Indirect export	59%
Through trade corporation	59%	Advertising	65%	Normally not	6%	Joint venture	12%
Through local agent	41%	Sales literature	59%	No	6%	Direct investment	0
Through retailer	0	Word of mouth	18%				

Qualitative research was used to triangulate the topic. The results from the interviews are categorized in those topics that were chosen for further research after analyzing the survey results.

1. To what extent do the registration barrier, Chinese internal competition and payment problem affect the business? All the interviewees feel that these three factors have affected the international business of Chinese firms to a very high degree. Internal price fighting is viewed as the biggest headache for overseas companies when dealing with the Chinese. Product registration has been seen as the first stage to go through, and the requirements in most of the countries remain very high. A low profit margin does not allow them to make more investment in foreign markets where high registration standards have been set; again high risks on long-term credit payment give more worry.

2. Will low cost continue to be a dominant advantage in the future? It was stated that low cost has been the unique advantage for Chinese firms in international business to get attention from overseas markets and a major threat to multinational corporations. This seems to continue its important role in the near future, but is not the only factor for

success. Chinese firms need to look at other elements rather than only relying on price. Significantly, some people are already seeing the trend of increasing costs when the company starts becoming larger and investing more on human resource and R. & D.

3. What kind of strategies should Chinese firms adopt in international marketing? More discussions have been made mainly on this question with all the interviewees. They all remarked that it is the time Chinese firms paid more attention to making efficient strategies in order to succeed in the global market. These need to be well planned based on own strength, weakness, opportunity in the international environment and competition in the market. The main options recommended are as follows:

    - Build up brand name
    - Have more entry modes
    - Strengthen low cost advantage
    - Provide more value-added service both commercially and technically
    - Accelerate new product development process through more investment on R & D
    - More investment on international human resource management to improve expertise

DISCUSSION

The best way to design strategies is firstly to understand the fundamental basis of various elements including their advantages and weaknesses. The following paragraphs give a comprehensive analysis of different strategies and scenarios involved in international marketing, and highlight specific opportunities and threats.

Carrying out comprehensive market research will cost more to companies in terms of funds, human power etc. But it does give an efficient account of information for marketing decision-making, and could help control the implementation of all the strategies in the structure of the market information system. Distribution in international marketing is always determined by the selected entry modes. At present, the exporting system used is directly to an importer and distributor/agent. However, adopting a strategic alliance with direct investment will lead firms not only to the importer but also the local retailer and end user. Working jointly with foreign customers could be a good alternative to share the know-how and resources with each other. By this process, Chinese companies will gain a better understanding of customer needs, culture and the adoption of marketing strategies in a foreign marketplace through advertising campaigns and sponsorship than sitting at home relying on direct export.

Along with the expansion of business, firms have to consider their own registration in overseas markets. It will help the company sell registered products to any local business party within the territory with more choice and controllability.

Trade corporations and manufacturers are two groups of Chinese firms in the international market. The main strength of trade corporations is trading skills and sales networks; manufacturer is good at production, new product development and technical service. Union of these two types of strength can improve the competitiveness for the Chinese

pesticide industry in the global marketplace rather than making spiteful internal competition.

In the economic theories, government normally keeps prices for all the raw material, labor and technology at a low level in order to support the labor intensive manufacturing industries at the initial stage of economy development, so that it will rapidly move the economy forward. Today's economy in China is growing with efforts made in last two decades (Foy & Maddison, 1999), and low cost has become the unique advantage. This trend will still continue in the near future. But again, theoretically thinking, along with the continuously growing economy, all the prices of raw material, labor, technology and environment (Smil, 1997) will definitely go up to some extent. Will we be able to sustain this unique advantage when that time comes?

Therefore, before this happens, strategically looking at the other factors for success in international marketing seems required for all the Chinese companies. Understand the fundamental concepts of different marketing strategies, analyze the firm's own strength, weakness, potential business opportunities and threat in the marketplace, and make a choice.

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Influence of *Pseudomonas oryzihabitans* on growth of tomato plants and development of root-knot nematode *Meloidogyne javanica*.

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ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) constitute one of the major pests, causing significant economic loss to agricultural crops worldwide. Currently effective management depends on chemical treatments. Methods based on biological control are needed to reduce the threat of environmental damage. The bacterium *Pseudomonas oryzihabitans*, symbiotically associated with *Steinernema abbasi*, was shown to produce compounds with nematostatic effects *in vivo*. Roots of tomato plants were dipped in suspensions of *P.oryzihabitans* at different concentrations, which were later inoculated with nematodes. In treatments at 10^4 and 10^5 cells ml^{-1}plant growth was improved and nematode development delayed.

INTRODUCTION

Crop production has steadily increased over the decades primarily through the use of fertilizers and chemical pest control. However, plant parasitic nematodes can cause crop losses of from 5-12% annually. Moreover, in modern agriculture scientists and farmers need to consider how pesticides may affect non-target organisms in soils. Chemical treatments may need to be applied several times over one growing season (Johnson & Feldmesser, 1987). There is an increasing interest in applying microorganisms to control plant pests and diseases as a part of alternative IPM strategies. Among the bacteria, which colonise the root system, fluorescent pseudomonads have received particular attention because of their ability to produce a wide range of antifungal metabolites. The bacterium *Pseudomonas oryzihabitans*, is widely distributed having been isolated from soil samples in UK (Elinor, 1999).

MATERIALS AND METHODS

The bacterium *P. oryzihabitans* was grown in a Nutrient Broth No2 shaken for 48 hours at 28 °C and then centrifuged in 250 ml tubes at 4100 rpm for 17 minutes. Tomato plants c.v. 'Money Maker' were grown in 9 cm diameter plastic pots filled with John Innes No2 loam based compost. Each tomato plant, at either 5 or 12 days after germination, was removed from the pot and the root systems were thoroughly washed with sterilised water and dipped into a suspension of *P. oryzihabitans* at concentrations of 10^3, 10^4, 10^5, and 10^6 cells ml^{-1} for 3 min. Plants were then re-planted in the same pot and one day later 100 and 500 juveniles (J_2s) of *M. javanica* were added to these pots. Root systems of untreated plants were dipped in

distilled water for the same period of time and other plants were inoculated only with the required numbers of nematodes. All plants were kept in a glasshouse at 24-32 °C and treatments were replicated 10 times. After 32 days the plants were taken from the pots, the roots thoroughly washed free of soil and fresh root weights determined. Roots were stained with phloxine B and egg masses on roots counted (Al-Hazmi & Sasser, 1982).

RESULTS

Employing the Genstat 6. statistical programme, correlation analysis was performed for top fresh and root weight to assess inter-relationships. The two measurements were highly correlated assessed at 0.976. Top fresh weights were used to estimate plant growth and differences between treatments. ANOVA test was applied to assess differences between treatments and identify statistical differences between means.

Table 1. The effects of root dip treatments in different cell concentrations of *Pseudomonas oryzihabitans* on growth of 5 and 12 day old tomato plants inoculated with 100 or 500 juveniles (J_2s) of *Meloidogyne javanica*.

5 days old plants

Treatments	Top Fresh Weight		Probability*
	100 J_2s applied	500 J_2s applied	
Untreated plants	2.46 (±0.042) b	2.46 (±0.042) e	-
No cells applied	1.59 (±0.054) a	0.68 (±0.032) a	< 0.001
10^6 cells ml^{-1}	2.22 (±0.046) b	1.54 (±0.058) d	< 0.001
10^5 cells ml^{-1}	1.63 (±0.052) a	1.26 (±0.061) cd	< 0.001
10^4 cells ml^{-1}	1.73 (±0.026) a	1.06 (±0.034) bc	< 0.001
10^3 cells ml^{-1}	1.58 (±0.030) a	0.83 (±0.024) ab	< 0.001
Probability**	< 0.001	< 0.001	

12 days old plants

Treatments	Top Fresh Weight		Probability*
	100 J_2s applied	500 J_2s applied	
Untreated plants	10.18 (±0.102) d	10.18 (±0.102) e	-
No cells applied	7.05 (±0.089) a	7.06 (±0.075) a	NSD
10^6 cells ml^{-1}	8.05 (±0.087) b	7.75 (±0.114) ab	NSD
10^5 cells ml^{-1}	7.72 (±0.153) ab	8.87 (±0.035) c	< 0.001
10^4 cells ml^{-1}	8.96 (±0.085) c	8.83 (±0.043) c	NSD
10^3 cells ml^{-1}	7.17 (±0.106) a	7.27 (±0.052) a	NSD
Probability**	< 0.001	< 0.001	

P value: * t-test between 100 J2s and 500 J2s across columns.

** Differences between values within columns. Values with the same letter are not different from each other.

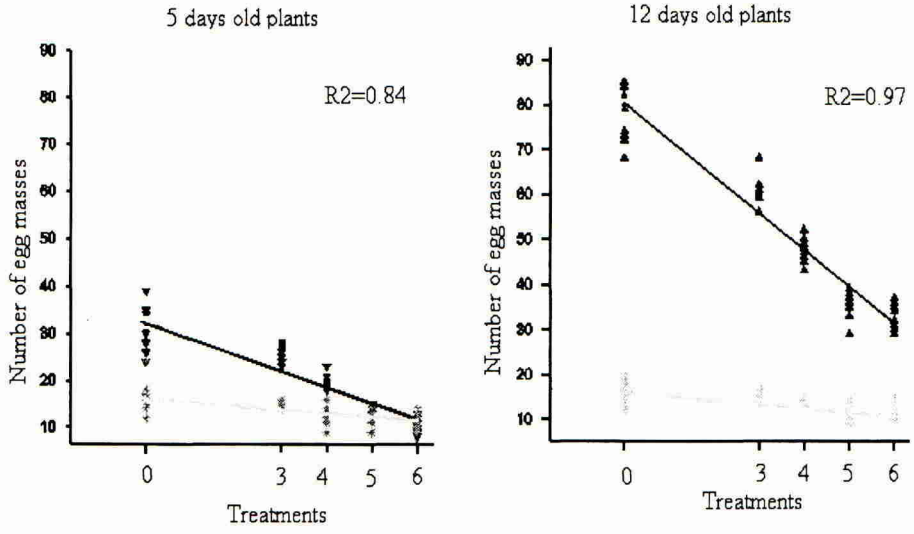

Figure 1.Regression equation for the relationship of the concentration of *P. oryzihabitans* cell suspensions with numbers of egg masses of *M. javanica* on roots, 32 days after replanting.

The root-dip treatments in cell suspensions resulted in an increase of plant growth particularly in the high nematode density treatment (Table 1). Top fresh weights of 12 days old plants inoculated with 500 nematodes/plant were promoted at 10^5 and 10^4 cells ml^{-1}, compared with other treatments. In addition, root-dip treatments in 10^5 and 10^6 cells ml^{-1} promoted top plant weight of 5 days old tomato plants. In terms of 5 days old plants inoculated with 100 and 500 nematodes, bacterial cells at concentration of 10^6 cells ml^{-1} significantly increased plant fresh weight. At the highest density, the bacterium clearly affected development and invasion of nematode resulting in fewer egg masses. This suggests that it either prevents nematode invasion or prevents the nematode development. However, at the low nematode density the slight effects were not significant (Figure 1).

DISCUSSION

This work indicates the possible activity of *P. oryzihabitans* as biological agent, against root-knot nematodes *Meloidogyne javanica* and its beneficial effects in tomato plant growth. Symbiotic bacteria from entomopathogenic nematodes are known to produce toxic metabolites (Akhurst, 1982; Chen *et al.*, 1994) because of their potential medical and

agricultural importance, entomopathogenic nematodes are now of great interest (Webster *et al.*, 1998). The results presented here provide evidence that the bacterium *P. oryzihabitans* produces metabolites, which have nematostatic effects. Nowadays, most of the Pseudomonas strains recognised as having biocontrol potential produce antifungal metabolites of which phenazines, pyrolnitrin, 2,4 diacetylphloroglycinol, pyoluteorin, viscosinamide and tensin are the most frequently detected. It is worthy of mention that Pseudomonads may produce more than one phenazine, which is related with their broad spectrum antibiotic activity (Kerr, 2000).Studies in characterisation and in biosynthesis of these compounds are important to be conducted in future works.

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Ultrastructural and cytochemical observations of *Musa* spp. in relation to susceptibility to nematodes

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ABSTRACT

Comparative ultrastructural and cytological observations were carried out in *Musa* roots infected by *Radopholus similis* in order to identify the resistance mechanisms involved. Resistant and tolerant cultivars reacted to nematode invasion with an increase in phenolic cells, formation of hypertrophied cells around lesions and occlusion of sieve tubes by active deposition of callose (tyloses). In addition, resistant varieties had greater areas of lignified cell walls in the epidermis and fewer air lacunae in the middle cortex than the susceptible varieties.

INTRODUCTION

Radopholus similis is the most economically important and widespread nematode pest of *Musa*. It is a migratory endoparasite present in many tropical and subtropical regions and is responsible for the destruction of the root system. *R. similis* was seen to be mainly located in the cortical parenchyma and associated with various forms of host cell damage, including cytoplasm retraction and cell wall degradation (Blake, 1966). The progression of the migratory nematode within the infected root is associated with severe modifications in host cell organization. These modifications varied among banana cultivars and depended mainly on the plant's reaction to nematodes. Pinochet (1978) observed heavily stained globules in the cytoplasm of cortical cells in plantain infected by *Pratylenchus coffeae*. Valette *et al.* (1997) found the endodermal cells of the partially resistant cultivars to be coated by a thick suberized layer suggesting that the endodermis acts as an effective barrier to nematode progression into the vascular stele. Moreover, Fogain and Gowen (1996) stated that the presence of preformed phenolic cells and lignification are two of the most probable mechanisms of the resistance to *R. similis* occurring in *Musa* clones. The aim of this study was the ultrastructural investigation of *Musa* cultivars in order to identify modifications in the root structure related with resistance to nematodes.

MATERIALS AND METHODS

Plant material

Four cultivars representing different genomic groups were selected based on their level of susceptibility to *R. similis*. The following varieties were used: YgKm5 (AAA), FHIA 01 (AAAB), FHIA 03 (AABB) and FHIA 17 (AAAA), the letters in brackets stand for the

genome of each variety. Five plants for each variety growing in 1 litre pots were inoculated with 5,000 *R. similis* axenically produced on carrot discs and five non-inoculated used as controls. Banana plants were harvested two months after inoculation.

Histology

Root tissues selected for microtome sectioning were fixed immediately in GDA (2% glutaraldehyde v/v) in 0.05 M phosphate buffer (pH 7). The fixed samples were washed in the buffer solution (3 times by 30 minutes each) and dehydrated in an alcohol series (30%, 50%, 80% and 80%). Infiltration was carried out using ethanol 80%: LRWhite resin (1:1), ethanol: resin (1:3), pure resin, 1 hour per change and finally pure resin for a week. Root pieces were embedded anaerobically in fresh resin in gelatine capsules and were placed in an oven (50 °C) for 24 hours for polymerisation. A series of 2 microns sections were taken from each sample with a historange microtome. The sections were stained with toluidine blue and safranine and were then mounted in DPX. The slides were scanned under light microscope and image analysis was made with the use of scion imaging software.

The experiment was a completely randomized factorial design with two treatments and four varieties.

RESULTS

General observation

All the varieties used in this experiment have the same structure. No differences were observed between nematode-infected and control plants in the cross section cell organization (Table 1). As revealed from the toluidine blue-safranine staining, lignified cells were located in the epidermis, endodermis and vascular cylinder in both infected and control plants. No lignified cell was found in the cortical parenchyma of either resistant or susceptible cultivars. In addition, in FHIA 03 tyloses and hypertrophied cells formed only in the nematode-infected plants (data not presented).

Dermal tissue

There was no effect of the interaction between variety and treatment on the cell area, cell wall and dermal tissue thickness (Table1). Varieties differed in the cell size in this area. The tetraploid varieties had larger cells but thinner cell walls than the triploid variety. FHIA 03 had the largest cells and YgKm5 the smallest. In contrast, YgKm5 had the thickest walls and FHIA 03 the thinnest. FHIA 01 and FHIA 17 had the same cell area and cell wall thickness. The dermal tissue consisted of 2 to 3 rows of cells, thus the extent of this area varied among species. FHIA 01 had thicker epidermis than YgKm5 and FHIA 03.

Cortex

There was no effect of the interaction between variety and treatments on the cell area, cell walls thickness and the length of the cortex area. Varieties differed in the length of the outer cortex. In FHIA 01 the outer cortex covered greatest cross sectioned area and differed from the other FHIA varieties and YgKm5. FHIA 03, FHIA 17 and YgKm5 had the same area.

There were no differences between varieties in the cell size and cell wall area but in the inner cortex, the cell size and cell walls did vary among varieties. FHIA 17 had larger cells and differed from FHIA 01 and YgKm5. On the contrary, YgKm5 and FHIA 01 had thicker walls than FHIA 17. FHIA 03 had similar cell area and wall thickness with FHIA 17. Nematodes did not affect the cell area and wall thickness of the cortex (Table 1).

Aerenchyma

The effect of the interaction between variety and treatment on the cross section area occupied by aerenchyma was not significant. Varieties differed. FHIA 17 had the largest aerenchyma in the cortex and FHIA 01 the smallest (Table 1). YgKm5 had similar aerenchyma to FHIA 03. Nematodes had no effect on the formation of the aerenchyma.

Table 1. Differences between nematode-infected and control plants on the cross section area occupied by aerenchyma, cell area and cell wall thickness (μm^2 / μm^2 of section area)

Cultivars	Level of suscep.	Aerenchyma	Cell wall thickness			Cell area		
			Dermal tissue	Outer cortex	Inner cortex	Dermal tissue	Outer cortex	Inner cortex
			Control plants					
FHIA 01	r	0.080	0.331	0.252	0.233	0.669	0.748	0.717
FHIA 03	t	0.119	0.299	0.228	0.212	0.701	0.772	0.729
FHIA 17	s	0.144	0.349	0.229	0.184	0.650	0.772	0.761
YgKm5	r	0.087	0.397	0.270	0.236	0.605	0.730	0.720
			Nematode-infected plants					
FHIA 01	r	0.064	0.325	0.245	0.218	0.680	0.755	0.729
FHIA 03	t	0.104	0.336	0.266	0.203	0.664	0.734	0.745
FHIA 17	s	0.147	0.365	0.193	0.186	0.635	0.807	0.764
YgKm5	r	0.106	0.392	0.228	0.209	0.608	0.772	0.744
SED (0.05)		0.016	0.024	0.023	0.019	0.023	0.023	0.020

s=susceptible, r=resistant, t=tolerant

Phenolic like substances

Phenolic like substances occurred in the cross sections of all varieties. They were mainly located in the stele and only few are scattered in the cortex. There was an interaction between variety and treatment on the number of phenolic cells. In FHIA 01 control plants had more phenolics than nematode-infected plants. In contrast, nematode-infected FHIA 17 plants had more phenolics than the controls. In FHIA 03 and YgKm5 there were no differences in the number of phenolic cells between nematode-infected and control plants. Overall, FHIA 01 and FHIA 17 had greater numbers of phenolic cells than FHIA 03 and YgKm5 (Figure 1).

DISCUSSION

This study shows that there are root characteristics related with plant resistance to nematodes. The lignification and the thickness of the epidermis could be one of the resistance mechanisms

as the susceptible varieties appeared to have a smaller dermal cell wall area than the resistant variety YgKm5. Another factor involved in the susceptibility-resistant response is the phenolic compounds. This investigation showed that phenolics are associated with the defence strategy of banana to *R. similis* infection. However, this is not the only mechanism since the resistant variety FHIA 01 had fewer phenolics in nematode-infected plants than the susceptible varieties. Differences were also found in the size of air canals. Aerated conditions favour nematodes multiplication in roots; thus cultivars with oxygen abundance will provide the required environment for nematode multiplication and spread.

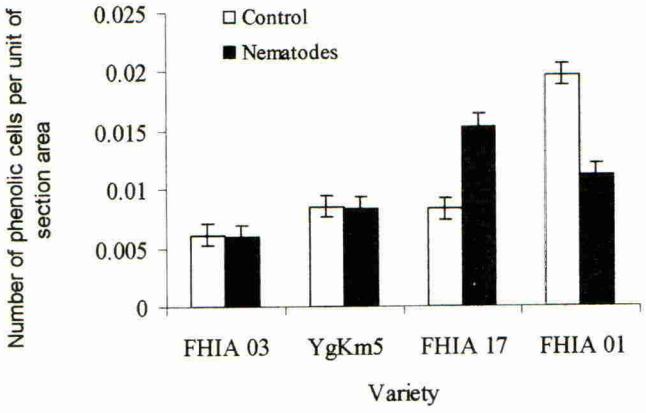

Figure 1. Effect of nematodes on the total numbers of phenolic cells in different banana varieties

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Crude protein and lipid concentration in grains from oats infected with barley yellow dwarf virus

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ABSTRACT

A field experiment was conducted south of Uppsala, Sweden, in 2002. Oat plants were artificially infested at growth stage 11, 13, 31 or 39 with aphids (*Rhopalosiphum padi*) infected with barley yellow dwarf virus (BYDV). An uninfected control was also included. After harvest, crude protein, lipid, Ca, P, K and Mg contents in the grains were analysed. The grain crude protein concentration was significantly higher after all infestation growth stages, and the lipid concentration significantly lower in grains from the plants infested at growth stage 11, 13 and 31, than in grains from the control plants. There were no statistically significant differences in crude protein concentration and lipid concentration between the infection times.

INTRODUCTION

Barley yellow dwarf virus (BYDV) is economically damaging in cereals and grasses worldwide (Lister & Ranieri, 1995). As well as causing reductions in above-ground biomass (D'Arcy, 1995) and grain yield (Lister & Ranieri, 1995), BYDV infections may also give rise to higher grain protein concentrations (Potter, 1980, 1982; Edwards *et al.*, 2001).

During a period of senescence, proteins involved in photosynthesis are broken down to amino acids, transportable from photosynthesising tissues to e.g. grains (Broquisse *et al.*, 2001). The senescing agent potassium iodide (KI) has been applied to plants to induce drought stress senescence in cereal plants (Fernandez-Figuares *et al.*, 2000) and resulted in an increased concentration of grain protein due to a lack of carbohydrates and to enhanced protein hydrolysis. An induced senescence does not affect the plant nitrogen content to the same extent as the carbon content since approximately 75% of the nitrogen in a cereal plant is taken up before growth stage 39 (GS 39), whereas approximately half of the dry matter production based on CO_2-assimilation takes place after GS 39 (Hay & Walker, 1989). With KI induced senescence, there is also a negative relationship between carbon and nitrogen translocation from photosynthesising tissues to grains (Fernandez-Figuares *et al.*, 2000).

The discoloration of BYDV infected leaves is a result of chlorophyll degradation (Jensen & D'Arcy, 1995) and can be regarded as advanced senescence. Analogously to KI-induced senescence, BYDV-induced senescence could entail an increased nitrogen concentration in the plant and a higher grain protein concentration. However, since about 50% of the nitrogen in a plant is taken up between GS 30 and 39 (Hay & Walker, 1989), BYDV infection before GS 30 could probably also disrupt nitrogen uptake, with effects on plant nitrogen status and grain protein status. In addition, leaf discoloration intensity and area depend on the growth stage in

which plants are infected (Doodson & Saunders, 1970), suggesting that the growth stage in which the infection takes place is vital to the grain crude protein concentration. The main objective of the present experiment was to quantify differences in grain crude protein concentration after infections with BYDV at different plant growth stages.

Another objective was to quantify the effects of BYDV infection on grain lipid concentration. We expected a decreased lipid concentration as a result of the advanced senescence caused by the BYDV infection, since oats with shorter growing periods have lower grain lipid concentrations (Saastamoinen *et al.*, 1989).

MATERIALS AND METHODS

Oats (*Avena sativa* cv. Stork) were sown on 29 May 2002 at a site south of Uppsala (59°58´N, 17°35´E) on an experimental area of 24m x 40m. The experiment, with a randomised complete block design, consisted of five different treatments in which oat plants were either infested with viruliferous aphids (*Rhopalosiphum padi*) at GS 11, 13, 31 or 39 or left uninfected (control). Five wood framed cages covered with transparent fine-meshed synthetic netting and a volume of $1m^3$ each were randomly distributed in each block to protect the experimental plants from naturally occurring aphids.

The oat plants were infested by placing leaves with approximately 15 viruliferous aphids at a distance of 3-5 cm along the three crop rows containing the largest number of plants within the cages. No edge rows or rows with long gaps without plants were chosen. Six days after the start of the aphid infestations, the oat plants were sprayed with the insecticide Pirimikarb. The roofs of the cages were removed after the last spraying. Visible gradations and TAS-ELISA tests were carried out to assess the BYDV infection. When observed, frit flies, rust and mildew fungi were treated with recommended pesticides. Weeds in the cages were removed by hand.

Plants from the three infested rows and from the three rows in the control cages that contained the largest number of plants were harvested at GS 87 and dried. After threshing and cleaning the grains were weighed. Dry weight values were scaled to an area basis by multiplying weight per plant by number of plants per area. Whole grains, hull and bran included, were analysed for crude protein, lipid, Ca, P, K and Mg content. Crude proteins were determined by the Kjeldahl method (Anonymous, 1993) (hydrochloric acid used in the titration instead of sulphuric acid and the distillate collected in boric acid). The lipids were analysed according to Anonymous (1998) procedure B (hot extraction used instead of soxhlet). Ca, P, K and Mg were analysed with an automated micro-digestion method according to Andersson (1996).

Differences in chemical composition between treatments were statistically tested with an analysis of variance and Tukey's pairwise comparisons with a family error rate of 0.05. Values from one cage in the GS 13 treatment were excluded from the statistical analysis due to an incorrect counting of the number of plants in that cage.

RESULTS AND DISCUSSION

Crude protein concentration was significantly higher in the infected plants than in the control plants (Table 1). This increase in crude protein was in accordance both with predictions based on senescence studies and with results from other studies (Potter, 1980, 1982; Edwards *et al.*, 2001). There was no statistically significant effect of GS at infestation on crude protein concentration (Table 1), probably due to similar effects on leaf protein degradation and nitrogen translocation after all infection stages. Grain crude protein content (g protein/m^2) was lower for the earliest infections than for the later, probably due to the lower N demand for low growth rate plants (Touraine *et al.*, 2001). The total crude protein content in the plants infected at GS 39 was higher than that in the control plants, although not statistically significant. One possible explanation for the increased crude protein content might be a higher exhaustion of nitrogen in the vegetative parts of the infected plants than in the uninfected plants, which is in line with the negative relationship between nitrogen and carbon phloem transport shown by Fernandez-Figuares *et al.* (2000).

The lipid concentration was significantly lower in the GS 11, 13 and 31 treatments than in the control (Table 1), in agreement with earlier studies showing that a shorter growing period is related to a decreased lipid concentration (Saastamoinen *et al.*, 1989).

Table 1. Mean values of biomass (g d.m./m^2), crude protein, lipid and mineral concentrations (weight %), and protein and lipid contents in the grains (g/m^2)

Inf. GS	Grain Biomass	Cr.protein conc.	Cr.protein cont.	Lipid conc.	Lipid content	Ca conc.	P conc.	K conc.	Mg conc.
11	204.5a[1]	12.1b	24.7a	5.28a	10.8a	0.83a	4.75a	8.70c	1.23a
13	289.2ab	12.7b	37.1ba	5.30a	15.5a	0.73a	4.80a	8.23bc	1.17a
31	492.5bc	12.9b	63.7bc	5.25a	26.0ab	0.63a	4.70a	7.48ab	1.22a
39	647.7c	12.1b	79.0c	5.43ab	35.1bc	0.68a	4.58ab	8.30bc	1.20a
control	709.7c	9.85a	69.4bc	6.03b	43.0c	0.65a	4.10b	6.95a	1.18a

[1] Different letter means a statistically significant difference between the treatments on a 0.05 family error rate basis.

CONCLUSIONS

The effects of BYDV infection in oats at different growth stages were investigated. The increased proportion of crude proteins and the decreased proportion of lipids in relation to grain biomass of the BYDV-infected plants are in line with previous results of experimentally induced senescence with potassium iodide. However, plant growth stage between GS 11 and 39 at which BYDV infection occurred had no significant effect. The effects of BYDV on dynamic crop growth have not been well examined. A further experiment in which the disease progression, in the form of diminished green leaf area, plant nitrogen content, plant size and plant dry weight, was measured under controlled conditions in a climate chamber would help clarify the infection effects. Such an experiment would also better explain why there were only small differences between the times of infection concerning the crude protein and lipid concentration.

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Control of brassica clubroot disease using modern fungicides possessing anti-protozoal activity

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ABSTRACT

Effective environmentally friendly control of brassica clubroot disease remains elusive. Liming to increasing the soil pH/calcium status is the only means available to UK brassica growers to suppress the damage caused by this pathogen. Our investigations have demonstrated that three fungicides, cyazofamid, fluazinam and fenamidone + mancozeb possess the ability to effectively control brassica clubroot disease. Effective control of gall development, (reducing inoculum build-up) was achieved by applying fungicides around the root system of the plant as a soil drench after transplanting.

INTRODUCTION

Clubroot disease is caused by the protozoan organism *Plasmodiophora brassicae* and affects a wide range of crucifers both of commercial and ornamental value, as well as some weeds. This disease is considered to be reaching epidemic proportions in the UK, where effective control methods remain elusive. The persistence of the resting spore stage of this pathogen, around 20 years, is a key obstacle to its eradication.

In the UK brassica growers achieve limited control of clubroot disease by liming their soils to increase soil pH and calcium levels. Dixon (1999) advises that races of *P.brassicae* have been identified in Lincolnshire (an intensive brassica producing area), which are able to thrive in alkaline soils. Screening investigations have identified fungicides that possess anti-protozoal activity, therefore the ability to effectively control clubroot disease. The active ingredients are cyazofamid (Ranman Twinpack, BASF), fluazinam (Shirlan, Syngenta) and fenamidone + mancozeb (Sonata, Bayer CropScience). All three fungicides have recently received approval for control of potato blight (*Phytophthora infestans*) in the UK, however it is important to note that Australian brassica growers have approval to use fluazinam, although application needs to be exact to reduce phytotoxic effects (Porter, *et al.*, 1998).

An experiment was conducted to generate further data of the efficacy of the three fungicides, assess their performance when applied at various rates, using two application techniques and challenged with two isolates of *P. brassicae* possessing differing virulence characteristics.

MATERIALS AND METHODS

A pot-based experiment was conducted within the controlled environment of a glasshouse (18°C day – 12°C night). Chinese cabbage (*Brassica rapa pekinensis*) cv. 'Yamiko' (Nickerson-Zwaan) was selected for this experiment because it is very susceptible to clubroot disease. Seedlings used for transplanting were raised using 60 cell modular plug trays filled

with Westland Seed Compost. The plug plant transplants were of a suitable size for transplanting after a period of 21 days from sowing. Plug plants were used to reflect current commercial brassica growing practices. Each treatment consisted of 10 x 10cm round deep profile 'Long Tom' plastic pots (BEF Growers), filled with John Innes N°2 loam based compost. Deep profile pots were selected in order to provide a deeper root run for the brassica plant. A brassica plug-plant seedling was transplanted in each pot. The fungicides were applied using two differing techniques, either by a pre-planting plug-plant dip (immersing the plug root system in a suspension of the fungicide) or by post-planting soil drench. Dosage rates are detailed in Table 1.

Table 1. Fungicide dosage rates and method of application

Fungicide & method of application	Rate per plant		Carrier per plant
	Premium	Reduced	
Control (*P. brassicae* inoculated)	-	-	100 ml H_2O per plant
Cyazofamid post-planting soil drench	0.08g a.i.	0.04g a.i.	100 ml H_2O per plant
Cyazofamid pre-plant plug dip	0.04g a.i.	0.02g a.i.	10 ml H_2O
Fluazinam post-planting soil drench	0.03g a.i.	0.015g a.i.	100 ml H_2O per plant
Fluazinam pre-plant plug dip	0.03g a.i.	0.015g a.i.	10 ml H_2O
Fenamidone/mancozeb post-planting soil drench	0.125/0.4g a.i.	0.06/0.2g a.i.	100 ml H_2O per plant
Fenamidone/mancozeb pre-plant plug dip	0.06/0.2g a.i.	0.03/0.1g a.i.	10 ml H_2O

Two isolates of *P. brassicae* were used, one collected from a field in Northern Ireland and the other from Essex. The Northern Ireland field isolate is extremely virulent while the Essex isolate is not significantly virulent in comparison. Inoculum was prepared as suspensions of resting spores of *Plasmodiophora brassicae* obtained by homogenizing root galls, previously stored at –20°C, in a Kenwood blender with deionised water for a period of 2 minutes. Homogenate was filtered through 8 layers of muslin cloth to remove coarse debris. Determination of spore concentration was made using an 'improved Neubauer' type haemocytometer. Each pot received a dose of 10^9 resting spores, (equivalent to 10^6 resting spores per gram of compost) in the form of quantified spore suspension.

After 40 days the experiment was harvested. The foliar parts of the Chinese cabbage were excised at soil level and dried at 80°C for 48 hours in order to determine the dry matter production over the growing period. Data were analysed by ANOVA, significant differences identified by Tukey multiple comparison tests.

The root systems were carefully washed out so that an assessment of gall development could be made. Gall development was assessed using a key described by Murakami, *et al.*, (2000). The root score data was converted into a disease severity index for each treatment using a formula reported by the same authors. Data were analysed using Kruskal – Wallis test, significant differences detected using non-parametric multiples comparison tests.

RESULTS & DISCUSSION

Dry weight data are shown in Figures 1 and 2. The cyazofamid and fluazinam fungicide treatments significantly increased the dry matter production of the Chinese cabbages that were

challenged with the highly virulent N. Ireland isolate of *P. brassicae* ($P<0.05$). The rate and method of applying cyazofamid and fluazinam fungicides, whether by soil drench or plug dipping appears to have little effect on the eventual dry matter production when challenged by a less virulent isolate of *P.brassicae*.

The formulation of fenamidone plus mancozeb caused a check to growth after transplanting (note the reduced dry matter production) and will delay maturity of crop (not assessed in this experiment). This aspect may reflect in brassica production schedules and may limit its use to crops were the harvest period is not critical, such as cabbage grown for winter storage.

Key to figures; **SD** – soil drench **PD** – plug dip : '**A**' – premium rate '**B**' reduced rate.

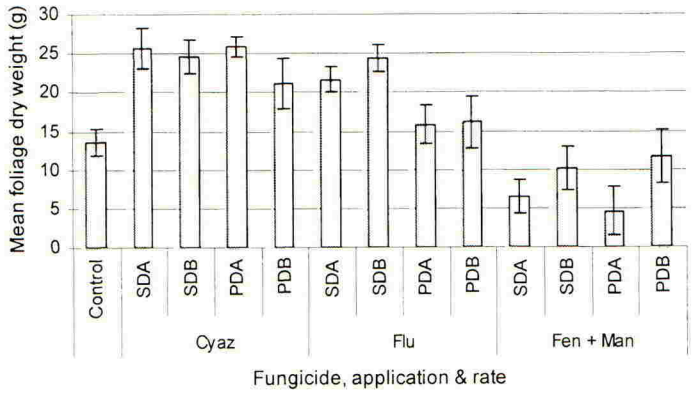

Figure 1. Mean foliage dry weight of Chinese cabbage heads when treated with fungicides to control N. Ireland isolate of clubroot. Error bars display 95% confidence intervals.

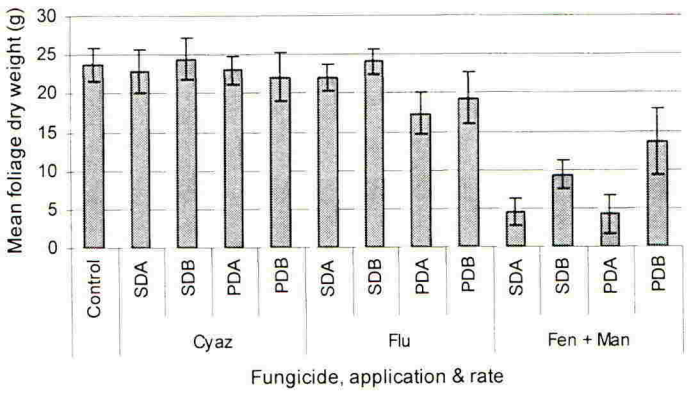

Figure 2. Mean foliage dry weight of Chinese cabbage heads when treated with fungicides to control Essex isolate of clubroot. Error bars display 95% confidence intervals.

The N. Ireland isolate of *P.brassicae* is highly virulent compared to the Essex isolate as disease severity associated with the former isolate is twice the later. The soil drenching method results in no or reduced galling of the root system (Table 2), thus minimising build-up of pathogen

inoculum. The higher volume of carrier associated with the soil drenching technique allows more effective wetting of the rooting zone compared to plug dipping. This experiment suggests the plug dipping technique reduces gall development enough to allow production of a respectable crop using only 50% of the fungicide requirement for the soil drenching method.

Table 2. Root system disease severity indices for Chinese cabbage

Fungicide & method of application	Rate	*P.brassicae* isolate	
		N. Ireland	Essex
Control (*P. brassicae* inoculated)	-	100	53
Cyazofamid post-planting soil drench	premium	0 *	0 *
Cyazofamid post-planting soil drench	reduced	10 *	0 *
Cyazofamid pre-plant plug dip	premium	27 *	10 *
Cyazofamid pre-plant plug dip	reduced	33 *	7 *
Fluazinam post-planting soil drench	premium	30 *	0 *
Fluazinam post-planting soil drench	reduced	23 *	0 *
Fluazinam pre-plant plug dip	premium	40 *	13 *
Fluazinam pre-plant plug dip	reduced	70	13 *
Fenamidone/mancozeb post-planting soil drench	premium	3 *	0 *
Fenamidone/mancozeb post-planting soil drench	reduced	16 *	3 *
Fenamidone/mancozeb pre-plant plug dip	premium	10 *	7 *
Fenamidone/mancozeb pre-plant plug dip	reduced	33 *	27

Note: * indicates significant reduction in disease severity compared to control ($P<0.05$)

The three fungicides of interest, cyazofamid, fluazinam and fenamidone possess similar modes of action, that being to inhibit mitochondrial respiration. Cyazofamid and fenamidone act upon complex 3 of the mitochondria whilst fluazinam operates at complex 1. This experiment demonstrates that the 3 fungicides are very effective for the control of clubroot disease and further work is continuing to assess their value in actual field crops.

ACKNOWLEDGEMENTS

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Mechanisms in the biological control of lentil vascular wilt (*Fusarium oxysporum* f.sp. *lentis*) by *Trichoderma hamatum*

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ABSTRACT

Biological control with microorganisms is a promising method for managing lentil vascular wilt. The efficacy of *T. hamatum* for control of *F. oxysporum* was investigated both *in vitro* and *in vivo*. *In vitro*, *T. hamatum* rapidly colonized and produced abundant conidia on *F. oxysporum* in a dual culture plate assay. Microscopic studies showed different hyphal interactions of effective *T. hamatum* towards the pathogenic fungus. The biocontrol fungus *T. hamatum* appears to be a mycoparasite of *F. oxysporum* hyphae. *In vivo*, soil treatment with *T. hamatum* significantly increased the population of *T. hamatum* to be effective and reduced the population of *F. oxysporum*. *T. hamatum* was a strong competitor, even at fairly low concentration, in the rhizosphere with *F. oxysporum* in loamy soil. The results of this study suggest that mycoparasitism and competition is one of the mechanisms involved in the inhibitory interaction of *F. oxysporum* with *T. hamatum*.

INTRODUCTION

Vascular wilt, caused by *Fusarium oxysporum* f.sp. *lentis,* is a major constraint to the production of lentil (*Lens culinaris* Medikus) worldwide (Saxena, 1993). It is the main yield-limiting factor in Syria, Morocco, Egypt and Ethiopia (Bayaa & Erskine, 1998). Microbial antagonism is an important factor for biological control of soilborne pathogens (Baker & Cook, 1974). Competition with microorganisms is considered to be one of the most important factors which determines the extent of the biological control activity of *Trichoderma* against soilborne plant pathogenic fungi (Papavizas, 1985). Rhizosphere competence is also important because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere. *Trichoderma* species either added to the soil or applied as seed treatments, grow readily along with the development of the root system of the treated plants (Zhang, *et al.*, 1996; Howell, *et al.*, 2000). The main objective of the present paper is to examine the hyphal interactions and study population densities of the phytopathogenic *F. oxysporum* and the biocontrol fungus *T. hamatum*.

MATERIALS AND METHODS

Fungal cultures

The biocontrol fungus *Trichoderma hamatum* (Bonord) Bainier (IMI 388876) was originally isolated from Syrian lentil soils and selected according to its *in vitro* antagonistic activity against *Fusarium oxysporum* (El-Hassan, 1998). *Fusarium oxysporum* f.sp. *lentis* (isolate no. 31) was obtained from stems of wilted plants collected from a sick plot at ICARDA.

Dual cultures

A single strain of *F. oxysporum* was challenged by *T. hamatum* on agar plates. The agar disks of the fungi, collected from the growing margins of fresh cultures, were inoculated at opposite sides (40-mm apart) of a 90-mm Petri dish containing potato dextrose agar (PDA) and the plates incubated in a growth cabinet at 25±2°C for 5 days in dark and 2 days under 12 h photoperiod. Mycelium intersections and subsequent overlap of hyphae of both *T. hamatum* and the pathogen began to form after 2-3 days. Ten replicate plates were used and the experiment repeated twice with similar results.

Scanning electron and light microscope for hyphal interaction

In order to investigate the antagonistic activity of *T. hamatum,* 10-mm dia. discs were excised from the interaction zone of 4 and 7-day-old dual colonies when the fungi were at their early stages of interaction. Mycoparasitic activities were observed with a light microscope (Olympus microscope-BH2, Japan). Mycoparasitic manifestations were photographed using an Olympus camera at x100 magnification. Some samples were also processed for scanning electron microscopy as described previously by Mycock and Berjak (1991).

Colony forming units determination for fungal colonisation

The experiment was conducted in 150-mm-dia. plastic plates containing sterilized sandy-loam soil treated with peat-*Trichoderma* inoculum under laboratory conditions. Soil-plates were stacked vertically in complete randomized blocks, covered with aluminium sheeting to prevent light and watered lightly before planting. Two surface-sterilized lentil seeds (ILL 4605) were sown in each plate; 15-day seedlings were inoculated with a 6 ml suspension ($2.5x10^6$ microconidia/ml) of *F. oxysporum* (Erskine & Bayaa, 1996) per plate. Fungal colony-forming units (CFU) in rhizosphere and infected plants were individually determined by placing serial dilutions of soil homogenate and 10-segment surface sterilised stems on TSM and Komada's agar medium (Komada, 1975; Askew & Laing, 1993). Fungal densities were quantified, by a plate-dilution technique on selective media, at 15-day intervals after planting and inoculation date. The experiment was set-up in a randomized block design with 4 replications. The population sizes of fungi were transformed to their $\log_{10}$ of CFU and the percentage values of plant *Fusarium*-infected to their square root (SQRT) before analysis.

RESULTS

Scanning electron and light microscope for hyphal interaction

Trichoderma hamatum completely inhibited and overgrew *F. oxysporum* 7 days after inoculating the agar plates (data not shown). Scanning electron and light microscopical observations of the interaction zone demonstrated clearly the following interaction responses: *T. hamatum* established close contact with the mycelium of the pathogen by coiling them densely and tightly, even at early stages of interaction (Figure 1, A), parallel and vaculize (Figure 1, B), climb (Figure 1, C) and penetrate the hyphae and strong compression of pathogen cells as illustrated by the wrinkled appearance of mycelium surface (Figure 1, D). In the later stages of parasitism, *F. oxysporum* hyphae showed extreme collapse (Figure 1, E). These observations support the findings that *T. hamatum* was not only able to inhibit the growth but, also, parasitize the hyphae of *F. oxysporum*.

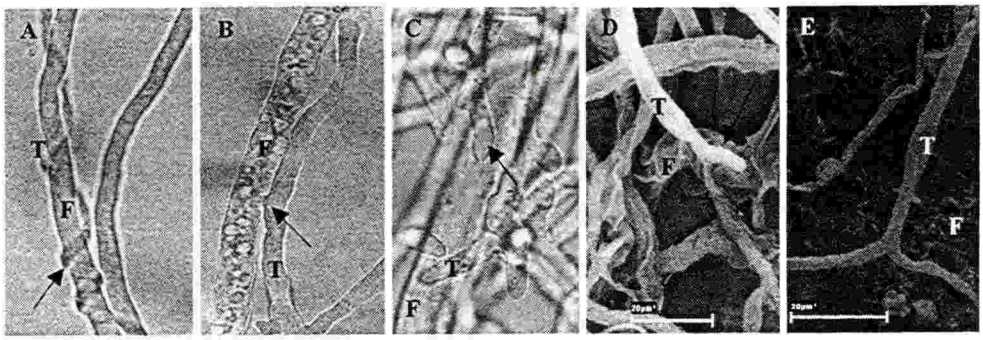

Figure 1. Mycoparasitism of *F. oxysporum* by *T. hamatum* within 7 days after inoculation, F: *F. oxysporum*, T: *T. hamatum*. **A**: light micrograph showing *T. hamatum* hyphae coiling around hyphae of *Fusarium* (1000x Mag), **B**: *T. hamatum* hyphal parallel and vaculize hyphae of *Fusarium*, **C**: *T. hamatum* hyphae tips climbing *Fusarium* hyphae, **D**: electron micrograph showing *T. hamatum* hyphae tips penetrating *Fusarium* hyphae (3000x Mag.), **E**: by 12 days, *Fusarium* hyphae collapse, where *T. hamatum* hyphae continue to look normal. Bars= 20 μm.

Colony forming units determination for fungal colonisation

Results have confirmed that *T. hamatum* survived well (either alone or with *F. oxysporum*) in soil under room conditions. The population of *T. hamatum* had rapidly increased up to 10^{10} (day 14 of *Trichoderma* and 0-day for *Fusarium* soil application) and slightly decreased to 10^4 CFU/g soil at the final sampling time, 56 days after planting (Figure 2, A). In the soil treatment with *T. hamatum,* the results clearly show that the reduction in densities of *F. oxysporum* recovered compared with control is probably related to a decrease in *F. oxysporum* population due to an increase in population of *T. hamatum* in the rhizosphere around the roots during the growth period (Figure 2, B). Overall, the soil treatment with different concentrations of *T. hamatum* significantly reduced wilt the severity on plants (Figure 2, B).

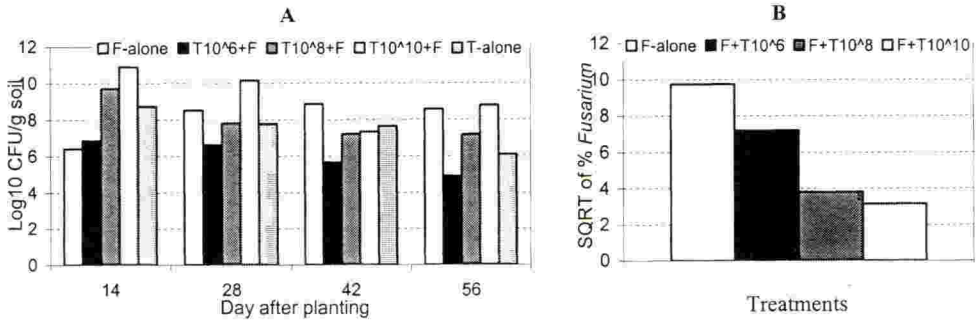

Figure 2: Population dynamics of *T. hamatum* and *F. oxysporum* and *Fusarium* colonisation of lentil planted in sandy-loam soil (F: *F. oxysporum* alone, T+F: *T. hamatum+F. oxysporum,* T: *T. hamatum* alone,). **A**: populations were determined by plate-dilution technique on fungal selective medium for each sampling date and the values are the means of 4 replications. **B**: fungal pathogen colonisation was expressed as a percentage of infected segments in each plant (10 pieces per stem).

DISCUSSION

The development of successful strategies for the use of biocontrol agents to protect the plant from fungal attack will require a detailed knowledge of the mechanisms employed by the biocontrol agents to effect control. Timing of application, application strategy and establishment of biocontrol agents at the target area are the critical elements for successful biocontrol (Baker & Cook, 1974). Nutrient competition is a potential mechanism for suppression of plant pathogens by endophytic fungi. Light and electron microscopical investigations of co-cultures confirmed the microscopic differences in hyphal interactions. These findings, in general, confirm previous studies made by using of *Trichoderma* sp. as a rhizosphere colonizer with biocontrol potential on various crops (Zhang, *et al.*, 1996; Howell, *et al.*, 2000). The results indicate that *T. hamatum* established well in soil which has a strong activity against the pathogen and reduced wilt incidence of lentil.

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Seed treatment with a bacterial antagonist for reducing cotton damping-off caused by *Pythium* spp.

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ABSTRACT

The potential biocontrol agent *Pseudomonas oryzihabitans* was used as seed treatment ('seed coating') and as a soil drench to reduce the incidence and severity of seedling diseases of cotton caused by *Pythium ultimum* and *P. aphanidermatum*. In the glasshouse the application of *P. oryzihabitans* cells to soil infested with *Pythium* spp. resulted in significant improvement of plant growth.

INTRODUCTION

With the increasing public awareness of the possible environmental implications of the use of pesticides in agriculture, alternative strategies for the control of plant diseases are being sought. Some of these strategies might involve the use of bacteria as biological control agents (BCAs). Compared to traditional control methods such as the use of fungicides or traditional cropping practices like crop rotation, biological control agents appear to be more environmentally friendly.

The bacterium *Pseudomonas oryzihabitans* has been shown to produce compounds that display anti-microbial effects (Vagelas *et al.*, 2001). *Pythium ultimum* and *P. aphanidermatum* are aggressive and rapidly growing soil saprophytes that may attack plants during their early stages of growth, causing seed rot, pre-emergence damping-off, post-emergence damping-off or stem rot. Losses occur in both greenhouse and field plantings and are more commonly seen during wet weather or in poorly drained areas of fields.

MATERIALS AND METHODS

Preparation of bacterial and fungal inoculum

The fungal isolates and bacterium strain tested were from the University of Reading collection. The isolates *P. ultimum* and *P. aphanidermatum* were cultured on potato dextrose agar (PDA) plates for 7 days. *P. oryzihabitans* cells were streaked on nutrient agar (NA) and incubated for 48 h at 28-30 ^{0}C. Cultures were then prepared by removing the colonies from the NA and placing them in nutrient broth solution for 48 h in shaking culture. Suspensions were standardized to approximately 10^6 colony forming units (cfu)/ml using a Spectronic 20 at an absorbance value of 0.1 at 600 nm. Other concentrations (10^3, 10^4 and 10^5 cfu/ml) were prepared by appropriate dilutions with sterile distilled water (SDW).

Seed germination assays

The efficacy of the bacterium for the biocontrol of *P. ultimum* and *P. aphanidermatum* was assessed in soil pot (1L) microcosms. A proprietary loam based compost (John Innes No 2) and infested at a 10:1 ratio with *Pythium* spp infested soil, to give a final concentration of approximately 10^4 oospores/g. A total of 60 g soil was added to each 150 mm Petri dish containing three 12.5 mm Whatman no. 1 filter discs. Cells were harvested by centrifugation and washed twice in SDW. Bacteria were inoculated directly to the system by applying 30 ml of a washed suspension directly as a soil drench, or by soaking cotton seeds (cv. Coker) in the washed suspensions for 10 min. Controls of water-treated soil and pathogen infected soil were placed directly onto the surface of the soil and experiment established in greenhouse conditions at 25 ^{0}C. Assessment was made 25 days after planting.

RESULTS AND DISCUSSION

The ability to inhibit the growth of a wide range of organisms is not common among biocontrol agents. Antagonists often have a high degree of specificity for a particular pathogen or strains of a pathogen (Kommedahl & Windels, 1980). However, *P. oryzihabitans* has been reported to control plant diseases caused by nematodes (Andreoglou *et al.*, 2001) and fungi (Kapsalis *et al.*, 2002), evidence that this bacterium has a broad spectrum of activity against microorganisms.

Table 1. Effect of seed treatments with *P. oryzihabitans* on plant shoot and root weights of cotton seedlings in soil infected with *Pythium* spp.

Treatments	Root weight [a]	Root weight [b]	Shoot weight [a]	Shoot weight [b]
P.ultimum	1.45a*	1.27a	4.22a	3.96a
P.ultimum / P. oryzihabitans 10^6cells/ml	2.62b**	2.84c	8.33b	8.18b
P.ultimum / P. oryzihabitans 10^5cells/ml	3.63c	2.45b	9.76b	9.09c
P.ultimum / P. oryzihabitans 10^4cells/ml	1.99a	1.67ab	5.19a	5.20a
P.ultimum / P. oryzihabitans 10^3cells/ml	1.68a	1.40a	4.50a	4.13a
P.aphanidermatum	2.20a	1.12a	4.23a	4.01a
P.aphanid. / P. oryzihabitans 10^6cells/ml	2.75b	2.74c	9.53b	7.56c
P.aphanid. / P. oryzihabitans 10^5cells/ml	2.66b	2.42bc	8.91b	7.18bc
P.aphanid. / P. oryzihabitans 10^4cells/ml	2.52b	1.48a	8.18b	5.84a
P.aphanid. / P. oryzihabitans 10^3cells/ml	2.39b	1.29a	4.65a	4.30a
Control	3.83c	2.95c	9.86b	9.27c

Seed germination assay with different bacteria formulations root drenching ([a]) and seed coating ([b])

* Evaluations were made at harvest (25 days after planting)

**Numbers in each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($P < 0.05$).

Disease caused by *P. ultimum* and *P. aphanidermatum* was significantly reduced when cotton seeds were treated by soaking in suspensions of the two higher concentrations *P. oryzihabitans* cells. There was a significant reduction ($P < 0.05$) in the disease development as measured by shoot and root weight, when seeds were treated with *P. oryzihabitans* compared to the

untreated control. Increasing the *P. oryzihabitans* concentration from 10^5 to 10^6 CFU (Table 1) per seed, significantly increased its efficacy to control the disease. These experiments suggest that seed treatment with the bacterium is a promising method for controlling seedling damping-off caused by *P. ultimum* and *P. aphanidermatum* and possibly other organisms causing seed rot and damping-off.

When cotton seeds were submerged in 4 different concentrations of *P. oryzihabitans*, root diseases were controlled by the bacteria colonizing the rhizosphere. Increasing the density of *P. oryzihabitans* cells on cotton seed would appear to provide better control of *P. ultimum and P. aphanidermatum.* In the drench treatments *P. oryzihabitans* had no critical effect on root weight of plants treated with *P. aphanidermatum* but there was significant effect on plant appearance treated with *P. ultimum* at the two higher concentrations of *P. oryzihabitans* . There was similar effect from the biocontrol treatment on shoot weights.

The higher cells concentration treatments (10^5 and 10^6) increased plant height ($P \leq 0.05$) compared with the other two treatments and that of untreated seeds in pathogen-infested soil (Figure 1). Plant heights from drenched soil treatments were greater ($P \leq 0.05$) than from the coated seed treatment for both pathogens. Similarly, coating seeds with *P. oryzihabitans* cells resulted greater plant height ($P \leq 0.05$) than in the control treated with *P. ultimum* and *P. aphanidermatum*

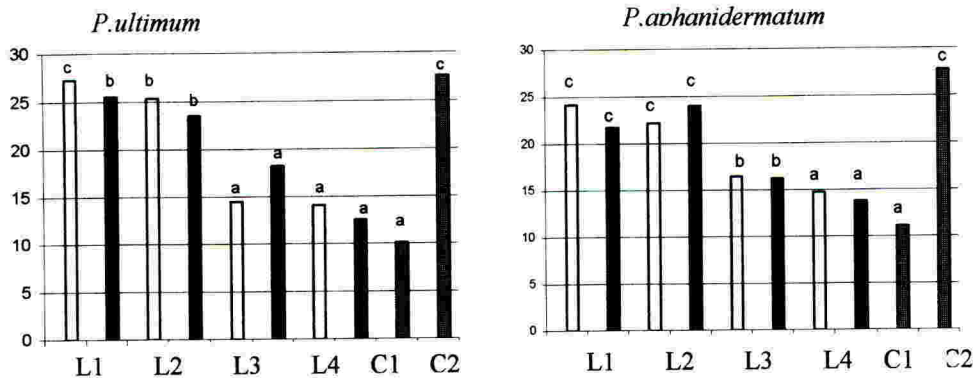

Figure 1. Effect of *P.oryzihabitans* seed treatments (seed drenching □ & seed coating ■) on heights of cotton plants growing in soil infected with *P. ultimum* and *P. aphanidermatum*. Inoculum levels L1 to L4 for *P.oryzihabitans* were 10^6,10^5, 10^4 and 10^3.cells/ml. The disease controls were C1 and cotton plant alone C2. Evaluations were made at harvest (25 days after planting). Means with the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($P < 0.05$).

Improved formulations and methods of applying this bacterium to seeds in soil may improve its efficacy as a biological control agent for damping-off diseases. Formulation may be the key for successful biocontrol agents because these organisms must be handled carefully to maintain viability through processing, storage, and application (Harman, 1991).

The application of *P. oryzihabitans* to cotton seeds led to effective control of damping-off caused by *Pythium* spp. under controlled environmental conditions. *P. oryzihabitans*

treatments efficiently control Pythium damping-off symptoms. Wet soil conditions are conducive to the development of *Pythium* spp. and other Oomycetes. More research should be done in different environmental conditions to investigate the potential of these novel biological control agents.

ACKOWLEDGEMENTS

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Disease control and the consequences of timing on the yield of oilseed rape

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ABSTRACT

Diseases of oilseed rape can account for substantial losses in yield in excess of 1 t/ha. Major UK diseases include phoma (*Leptosphaeria maculans*) and light leaf spot (*Pyrenopeziza brassicae*). The triazole fungicides tebuconazole and flusilazole can be used for effective control of these diseases. The timing of application of fungicides is important to control disease, limit yield loss and reduce costs. Optimal application programmes are therefore needed to satisfy these parameters. This paper summarises the results of two aspects, which are currently being investigated. Firstly, the development of GC-MS and ELISA extraction methods to detect flusilazole and tebuconazole residues in leaf material after spraying to determine biologically active concentrations of fungicides on leaves and their systemic activity. Secondly, a Burkard spore trap was used to collect samples from the field during the spring and summer of 2002 .A method for quantifying the inoculum levels of both *L. maculans* and *P. brassicae* that could be identified using PCR was developed. PCR was used to successfully identify 20 spores in a 20µl sample of spore suspension and samples from the field showed the presence of *P. brassicae* spores This information can be used to establish a link between spore presence in the field and the visible appearance of disease symptoms in the crop, thus allowing more accurate forecasting and precise spray applications. These investigations will elucidate the links between spray timing and control of oilseed rape diseases.

INTRODUCTION

Accurate identification of disease symptoms of *L. maculans* and *P. brassicae* are important in using the appropriate chemical and at the right time. Phoma leaf spot can be identified by brown lesions on leaves bearing pycnidia in the autumn with systemic growth leading to phoma stem canker later in the season. The triazole fungicide flusilazole can be used in autumn to control the leaf spot phase before the fungus grows down the petiole. Light leaf spot disease symptoms can be difficult to identify and often misdiagnosed as being due to frost or fertiliser scorch. Symptoms often cannot be identified visually until February or March but disease control decisions have to be made for effective control in November and December, often resulting in the unnecessary spraying or wrong timing. *P. brassicae* can be accurately diagnosed by incubating plants in polythene bags for four days and then assessed for the visible sporulation of the fungus (Fitt *et al.*, 1999).The length of time that the active ingredients in these chemicals are present is also an important factor to consider, as is any systemic activity they may have. GC-Ms can be used to determine pesticide residues of tebuconazole and flusilazole. Tebuconazole has also been detected in plant material using ELISA. Reduced rates of fungicides are often applied and there is some uncertainty as to the length of time these lower doses offer protection.

Polymerase chain reaction can be used to detect inoculum of several plant pathogens. Primers specific to *P. brassicae* and *L. maculans* (A and B isolates) have been developed, offering the potential for using PCR to monitor disease inoculum in the field. Air sampling using a Burkard seven day volumetric spore trap can give a visual assessment of inoculum present, but identification is time consuming and requires experience. *P. brassicae* ascospores and conidia are also very difficult to identify accurately. The combination of collecting samples from the field and detecting them using PCR is therefore a more accurate way of determining their presence or absence.

MATERIALS AND METHODS

Collection of spore samples in the field

A Burkard spore trap was placed in a trial of oilseed rape at Sutton Bonington in Leicestershire. The tapes were changed weekly and stored for visual identification of spores of *L. maculans* and *P. brassicae*. Samples were also stored for DNA extraction and detection using PCR.

PCR

The sensitivity of PCR to accurately measure small quantities of DNA extracted from spores is currently being investigated. DNA was extracted from melinex tape inoculated with spore suspensions and direct from spore.suspensions of known concentrations. Spore suspensions were made up to 20, 200 and 2000 spores per 20ml. The extraction method used was modified from a protocol published by Calderon *et al.* (2002) for extraction from the Burkard spore trap tape.

Inoculation of spore trap tape

L. maculans ascospores were collected from infected stems, which were soaked in water and placed in glass jars until ascospores were released. Spore suspensions were prepared and the concentrations calculated using a haemocytometer. *L. maculans* pycnidia were collected from 21 day cultures; plates were flooded with distilled water and the surfaces agitated with a sterile glass rod and the resultant spore suspension filtered through four layers of muslin. *P. brassicae* conidiospores were collected by placing infected leaves in sterile water and agitating for 20 seconds. Concentrations of both of these were then calculated using a haemocytometer. Melinex tape was prepared with a petroleum jelly mixture, (British Aerobiology Federation, 1995), and the tapes were inoculated with known concentrations of spore suspensions.

GC-MS

Extraction methods for ELISA and GC-MS are currently being investigated. Plants sprayed at field rates and half rates were harvested 48 hours after application. A 5g sample of plant material was ground in liquid nitrogen and then homogenised for 3 minutes in 40ml of acetone: water (3:1). An adapted method published by Maasfeld (1987) was then followed.

ELISA

Plants raised in controlled environment rooms were sprayed with full rate and half rate doses of tebuconaole and flusilazole. After 48h the plants were harvested and samples were extracted using an adapted method (Maasfeld 1987). NH-BSA conjugate, antisera and protocol were supplied by C Danks and used to determine levels of tebuconazole in extracted plant samples.

RESULTS

Two DNA extraction methods have been used to identify *P. brassicae* and *L. maculans* using PCR from spore samples attached to a Burkard spore trap tape with spore suspensions of a known concentration. PCR successfully detected the presence of both fungi from tape inoculated with 2000 and 20 spores per 20µl using an adapted method described by Calderon *et al.* (2002).

Conidiospores from *L. maculans* and *P. brassicae.*

A DNA extraction kit failed to yield appropriate amounts of DNA for PCR from inoculated tapes. The spores were adhered to the tapes, and previous workers have used Fastprep machines to remove the spores from the melinex tape. Specialist equipment was not available so other methods to remove and disrupt *P. brassicae* and *L. maculans* spores from the tape were investigated. Vortexing samples for short periods of time only resulted in DNA being extracted from high concentrations of spore suspension, 200-2000spores per 20µl. These levels would not be realistically seen on tape samples collected in the field so a more sensitive extraction method had to be developed. Various solvents were tried to wash the tapes in order to remove any spores attached, hexane was successfully used to show spores of both pathogens on the tapes after extracts were put through the PCR process. Hexane may however, inhibit other processes during PCR so this was not used on the field samples.

Finally, liquid nitrogen was used to grind samples attached to the melinex tape and then subjected to a DNA extraction using phenol:chloroform. This produced positive results on inoculated tapes and was then applied to the field samples. Field samples collected over the spring and summer of 2003 are now being processed and to date tapes have shown the presence of *P. brassicae spores* during June 2003 and often on consecutive days.

The extraction methods for GC-MS and ELISA are currently being investigated, it is hoped that these will determine any systemic and biological activity over time after spraying

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Integrated control of Fusarium ear blight

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ABSTRACT

Fusarium ear blight resistance was evaluated in three winter wheat cultivars under controlled environment conditions. The cultivars Rialto and Centrum exhibited resistance to initial infection whereas Charger was very susceptible to the disease. The spread of the pathogen was limited in the three wheat genotypes. The fungicide tebuconazole and the biocontrol agent *Bacillus subtilis*, were evaluated for curative efficacy in controlling FEB.

INTRODUCTION

Fusarium ear blight (FEB), caused mainly by *Fusarium culmorum* and *F. graminearum*, and the associated mycotoxin synthesis is a potentially serious problem in the UK and worldwide (Parry *et al.*, 1995). Control options for this disease are limited: highly resistant wheat varieties are not available (Bai & Shaner 1994), and current fungicides are not consistently effective. The most effective control of FEB is likely to be through an integrated approach including: selection of more resistant wheat varieties, applying foliar fungicides during anthesis, and applying biological control. Nonetheless, specific and consistent data are lacking in regards to which combinations of products and rates are most suitable for use in FEB management programmes.

In this study the resistance of wheat cultivars, fungicide and biocontrol efficacy against FEB were tested individually. Three winter wheat cultivars with varying degrees of resistance were assessed for resistance to initial infection and spread of the disease. Tebuconazole and a biocontrol agent, *Bacillus subtilis*, which have previously been shown to have antifungal activity against *Fusarium* spp., were evaluated for their efficacy in controlling FEB. This study provides the basis for further work on integrated control of FEB, which will involve testing different combinations of products, rates and timings for full control of FEB.

MATERIALS AND METHODS

The winter wheat cultivars Charger (susceptible), Rialto (moderately susceptible) and Centrum (resistant), which have been identified in the Monsanto screening programme, were used during the project. Seed of the winter wheat cultivars was sown into 12 cm diameter plastic pots containing John Innes No. 3 compost at a rate of 8 seeds per pot. Plants were germinated in a controlled environment at 15°C (day) and 12°C (night), with 16 h photoperiod for 10 days, transferred at 6°C for 8-9 weeks for vernalisation, moved at 15°C (day), 12°C (night) with 16 h photoperiod for 2 weeks and finally grown to flowering stage at 20°C (day), 15°C (night), with a 16 h photoperiod. All plants were regularly watered and treated against powdery mildew with quinoxyfen.

F. culmorum was sub-cultured onto PDA (Oxoid, Basingtoke, UK) and incubated under UV light at 20°C for 14 days. Conidial suspensions were obtained by flooding the surface of colonised PDA plates with sterile distilled water (SDW) and dislodging conidia using a sterile inoculating loop. The suspension obtained was then filtered through 2 layers of sterile muslin to remove hyphal fragments and centrifuged for 5 min at 1000*g*. The supernatant was decanted and centrifuge tubes were filled with SDW, shaken and centrifuged again. Number of conidia was adjusted after haemocytometer counts to reach a final concentration of 10^6 spores/ml in sterile distilled water. 0.01% of silwet L-77 was added to the conidial suspension just prior inoculation.

Two methods of inoculation were used to test the resistance of the cultivars: at anthesis (GS 61-69) heads were spray-inoculated with 2.0-3.0 ml spore suspension of 10^6 conidia/ml to test for reaction to initial infection; or tested by single floret injection into the floral cavity with a drop of spore suspension, to measure resistance to spread within the head. Sterile distilled water (SDW) was sprayed on heads or pipetted into the floral cavity of control plants. After inoculation, wheat plants were misted lightly with water and the heads were covered with plastic bags for 24 h. The trays were filled with water and the ears were sprayed with water daily in order to keep high moisture level. Heads were scored for FEB incidence (FEBI) (percentage of diseased heads) and severity (FEBS) (proportion of necrotic spikelets in affected spikes) 15 days after inoculation.

Tebuconazole (Folicur) and the biocontrol agent *Bacillus subtilis* (Botokiller, formulated for the control of botrytis diseases), were tested for curative efficacy in controlling FEB and toxin accumulation in grains. Wheat ears were spray-inoculated at mid-anthesis (GS 65), with 2.0-3.0 ml spore suspension of 10^6 conidia/ml of *F. culmorum*. The fungicide and biocontrol were applied at full field rate 3 days post inoculation. Control plants were sprayed with SDW. Pots were arranged in a randomised block design with 6 replicates. The disease was assessed visually 15 days after artificial infection. Thousand grain weight and DON toxin concentration in grain were also determined (data not shown).

RESULTS

Resistance

The two methods used to inoculate wheat ears with *F. culmorum* led to successful infection. Symptoms were already evident 7 days after inoculation.

Development of FEB varied among the different wheat cultivars following spray inoculation of *F. culmorum* at anthesis (Figure 1). Charger was susceptible to FEB, scoring 89.5% for FEBI and 73.4% for FEBS. In contrast Rialto and Centrum exhibited some resistance against initial infection. The FEBI for Rialto and Centrum were 59.1% and 66.7% respectively, and their FEBS were 40.6% and 35.9% respectively.

After point inoculation, the disease incidence was much higher than after spray inoculation. The FEBI for Charger, Rialto and Centrum were 100%, 90% and 81.2% respectively. This might be explained by the fact that the conidial suspension was directly pipetted into the floral cavity between the lemma and palea. The spread of the pathogen in the ears following point inoculation varied little among the cultivars tested. The three genotypes exhibited resistance

to spread. The FEBS in the cultivars Charger, Rialto and Centrum were 29.0%, 27.2% and 24.3% respectively.

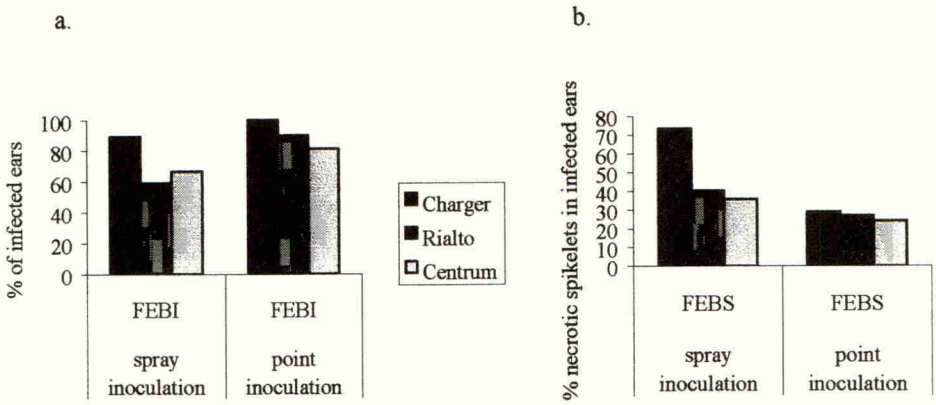

Figure 1. Disease incidence (a) and severity (b) in three wheat cultivars differing in resistance to FEB, 15 days after spray inoculation or point inoculation of *F. culmorum* (10^6 conidia/ml).

Schroeder and Christensen (1963) distinguished between two types of resistance to FEB in wheat: type I, whereby initial infection is inhibited, and type II, whereby, the spread of the pathogen in the host tissue is retarded. The three wheat genotypes exhibited type II resistance. Rialto and Centrum also showed some level of resistance to initial infection.

Antifungal activity of tebuconazole and *B. subtilis*

Ears were visually assessed 15 days after inoculation and the severity of FEB recorded as the percentage of spikelets showing symptoms. The effect of fungicide and biocontrol treatment on the severity of FEB caused by *F. culmorum* can be seen in Table 1.

Table 1. Effect of tebuconazole and *B. subtilis* applied at full field rate 3 days post artificial inoculation of ears at mid-anthesis with *F. culmorum* (10^6 conidia/mL) on the severity of FEB (* indicates significant difference with the control).

Cultivar	Treatment	% infected spikelets
Charger	Tebuconazole	13.1*
	B. subtilis	48.8*
	SDW	71.7
Rialto	Tebuconazole	7.3*
	B. subtilis	48.4
	SDW	60.2
Centrum	Tebuconazole	14.5*
	B. subtilis	54.8
	SDW	47.5

Applications of tebuconazole reduced significantly ($P<0.001$) the severity of disease in all three cultivars when compared to control sprayed with sterile distilled water. *B. subtilis* reduced significantly FEB symptoms in Charger ($P<0.001$) but there was no significant difference between treatment with *B. subtilis* and sterile distilled water in Rialto and Centrum. *B. subtilis* therefore failed to control *F. culmorum* in a curative strategy. After germination, the *B. subtilis* spores might face too much competition from an already established infection while the nutrients present in the formulation might feed the pathogen. Applying the biocontrol before artificial inoculation of the pathogen should allow the bacterial population to establish on the ears and be able to compete with *F. culmorum*. Further work will test prophylactic application of the biocontrol as well as the fungicide. Statistical analysis revealed no evidence of a significant difference between the cultivars treated with tebuconazole.

FURTHER WORK

This study presents the first step of research on integrated control of FEB. Fungicides and biocontrol need to be further tested in order to determine which rate, timing and combination will provide the best control of FEB.

ACKNOWLEDGEMENTS

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Suppressing weed competition: the interaction of seed quality and seed rate in spring wheat

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ABSTRACT

The importance of crop seed vigour and seed rate in integrated weed management of spring wheat was investigated in a field experiment at The University of Reading in 2002. In addition to the natural weed infestation, *Avena sativa* (spring oat) and *Sinapis alba* (mustard) were used as model weed species. Vigourous spring wheat seeds (germination > 98 %) suppressed above–ground weed biomass and increased crop yield significantly more effectively than less vigourous seeds (germination 87 % or 77 %). Doubling seed rate had no effect on weed suppression and only benefited a crop tolerant to weeds when sown with high vigour seed. Individual weed species displayed different responses to crop seed vigour. Weed reproductive output shows similar trend to weed biomass. The results demonstrate that with poor crop seed quality the use of increased seed rates will not achieve effective weed suppression.

INTRODUCTION

Weed management is one of the most significant challenges facing crop production, especially in organic farming. Herbicides are widely used by conventional farmers to tackle the problem of weeds. However, the desire to reduce herbicide reliance, cost of production, herbicide resistance as well as to respond to rising environmental concerns, have led to reconsideration of weed management tactics. Exploiting agronomic factors such as crop variety, seed rate, row spacing (Anderson, 1986) and early crop cover (Lemerle *et al.*, 2001) provide a potential to manipulate weed populations and suppress weed seed production. Despite much research on such factors, the role of crop seed vigour is still less well understood. This study was therefore designed to establish whether crop seed vigour, resulting from different seed ageing treatments, in combination with variable seed rate would affect weed suppression and crop tolerance to weed pressure.

MATERIAL AND METHODS

The experiment was conducted on the University Farm at Sonning, near Reading, Berkshire, UK, during the 2002 growing season. Using three vigour levels of the spring wheat cultivar Chablis (untreated and seeds aged for 38 and 48 h at 50°C), two seed rates (recommended and twice the recommended rate) and three weed treatments: naturally occurring weeds and as model weeds, *Avena sativa* var. Firth (oats) and *Sinapis alba* L. (mustard). The treatments were replicated twice in an incomplete randomised block design. Plots were 2x5m and drilled on 3 May 2002. Nitrogen was applied at 150 kg N ha^{-1}, with half the amount at sowing and the rest four weeks later. Fenpropimorph fungicide was applied on 25 June, photo-synthetically active

radiation was measured at regular intervals throughout the season in different canopy layers. Crop yields and weed biomass samples were collected from the central 1.0 m^2 area of each plot at the final harvest (Zadoks Growth Stage 92). Species were separated individually. Yield components of the crop, weed densities, weed biomass and reproductive output were estimated.

RESULTS

Yield

Effect of seed rate

The double rate yielded greater (P<0.001) than the recommended rate, but only for the high vigour seeds (Figure 1). However, increasing the seed rate of less vigorous seed showed a non-significant effect on yield, suggesting that doubling the seed rate failed to compensate for lower seed quality. The reason for that might relate to the slower emergence and poorer establishment of low vigour seeds.

Effect of vigour

In terms of crop's ability to cope with weed pressure, the low vigour plots performed poorly against all weed species (Figure 2). Conversely, in high vigour plots with mustard, there was a significant yield reduction (P<0.001) compared with other weed treatments. Obviously, crop tolerance to weeds varied with weed species. The relatively low grain yield of high vigour plots sown with mustard could be attributed to the uniform density and tallness of this weed that led to greater shading. Similar observations have been reported previously (Korres & Froud-Williams, 1997). Lemerle *et al.* (2001) also suggested that the outcome of competition for light among species is influenced by the relative height of the different genotypes.

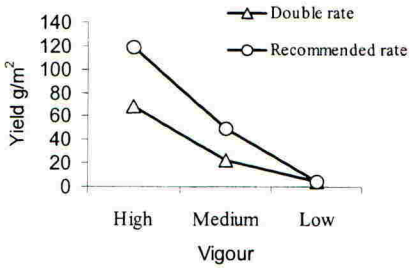

Figure1. Effect of vigour and seed rate on wheat grain yield.

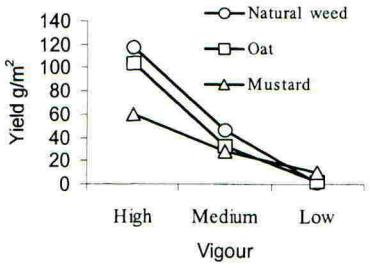

Figure 2. Effect of vigour and weed species on wheat grain yield.

Weed suppression

Effect of vigour

Significant effects of crop seed vigour ($P<0.01$) were obtained in weed suppression. Plots with high seed vigour gave high weed suppression, 28 and 31% reduction in terms of aboveground total weed biomass, compared to medium and low vigour plots respectively (Figure 3). However, the highest early emergence occurred at the high vigour. The reduction of naturally occurring weed dry matter was also significant, 35 and 40% respectively (Figure 3). *Anthemis cotula* (mayweed) was the most affected species. In comparisons of weed seed production, weed species varied in their response to crop seed vigour. Weed seed production was reduced by 84, 80 and 19 % in *Solanum nigrum* (black nightshade), *A. cotula* and *Viola arvensis* (field violet) respectively by the most suppressive vigour (data not shown). Weed seed production was very similar to weed dry matter in agreement with Wilson & Wright (1995). The lower weed seed production at high crop seed vigour means fewer weed seeds in the soil seed bank and in the harvested grain.

Effect of seed rate

Contrary to Grundy & Froud-Williams (1993) no suppressive effect of seed rate was observed for both total weeds and naturally occurring weeds. Samuel & Guest (1990) have also failed to report any detectable effect of increased seed rate on weed population, but did find an effect on weed biomass.

Effect of weed species

Although the main effect of weed species on the total weed biomass was non-significant, the effects on an individual basis and on the composition of naturally occurring weeds were highly significant ($P<0.001$: Figure 3). Naturally occurring weeds showed different responses to *A. sativa* and *S. alba*. Plots with *S. alba* reduced natural weed biomass by 50% compared to 13%

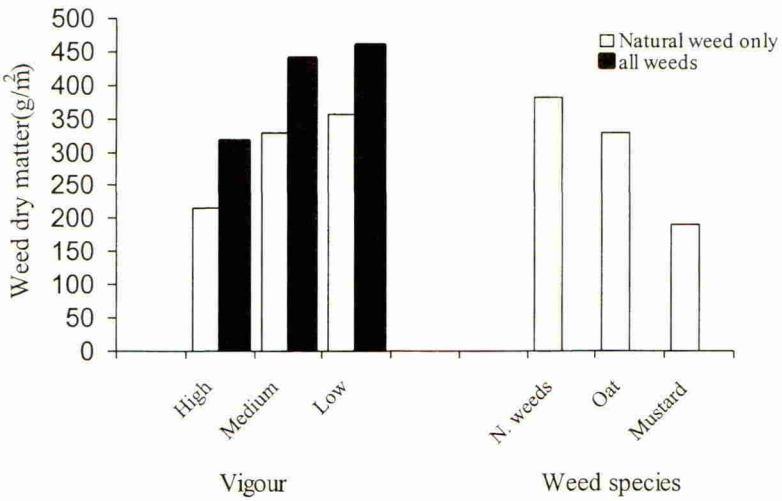

Figure 3. Effect of seed vigour and weed species on weed biomass composition

with *A. sativa*. *S. alba* found to be more than 20 cm taller than *A. sativa* and quicker in emergence. Tallness probably enabled *S. alba* greater shading on its understorey neighbours. However, there were no interactions between treatments in weed suppression.

DISCUSSION AND CONCLUSION

The results address the importance of seed quality as a component of integrated weed management. Although weed biomass varied with weed species, these differences were less marked than those caused by seed vigour. High vigour crop seeds suppressed weed biomass and this effect was probably due to the higher rate of emergence and earlier ground cover of the crop (data not shown). Lemerle *et al.* (2001) proposed that a crop species with a rapid rate of early growth is likely to have a competitive advantage over its weed neighbours.
In addition, the results from this work suggest that compensation for poor seed quality by drilling at a higher seed rate may not achieve any benefit in weed suppression, especially with the taller weeds

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