SESSION 5A

NEW COMPOUNDS, FORMULATIONS AND USES FOR DISEASE MANAGEMENT

Chairman: Dr D D Slawson,
Plant Health & Seeds Inspectorate, York, UK

Session organiser: Dr T M O'Neill,
ADAS Arthur Rickwood, Ely, UK

Papers: 5A-1 to 5A-5

Picoxystrobin : a new strobilurin fungicide for use on cereals

J R Godwin, D W Bartlett, J M Clough, C R A Godfrey, E G Harrison, S Maund
Zeneca Agrochemicals, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6ET, UK

ABSTRACT

Picoxystrobin is a new strobilurin fungicide discovered and developed by Zeneca Agrochemicals for use on cereals. It provides broad spectrum disease control in wheat, barley, oats and rye, demonstrating both preventative and curative activity under field conditions with a good persistence of effect. Picoxystrobin is unique amongst broad spectrum cereal fungicides in combining xylem systemicity with vapour activity, thereby ensuring optimum redistribution following application and helping to deliver its outstanding breadth of spectrum. Laboratory and field data have proven its very good safety and environmental profile. Excellent green leaf area, yield and quality benefits are delivered following treatment with picoxystrobin.

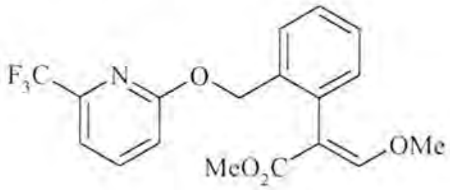
INTRODUCTION

The strobilurins are a class of fungicides whose discovery was inspired by naturally occurring fungicides produced by a range of wood-rotting fungi, e.g. *Oudemansiella mucida*. The discovery of the first commercialised strobilurins created great excitement because of their novel biochemical mode of action, low use rates and the broad spectrum activity of azoxystrobin in particular (Godwin *et al.*, 1992). Since their market introduction in 1996, strobilurins have established themselves as key components in crop protection programmes worldwide. Indeed, azoxystrobin was the world's leading proprietary fungicide in 1999 with sales of $415M. Picoxystrobin is the second strobilurin fungicide discovered and developed by Zeneca Agrochemicals. Although picoxystrobin has the same ß-methoxyacrylate moiety as azoxystrobin and the naturally occurring strobilurins, it has a different 'chemical backbone' which confers different physico-chemical and efficacy properties. Picoxystrobin has been selected for development because it demonstrates all of the ideal properties for a specialist broad spectrum cereal foliar fungicide.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name (IUPAC)

Methyl (*E*)-2-{2-[6-(trifluoromethyl)pyridin-2-yloxymethyl]-phenyl}-3-methoxyacrylate



Molecular formula : $C_{18}H_{16}F_3NO_4$
Molecular mass : 367.3
Physical state (at 20°C) : Solid
Melting point : 75°C
Density (at 20°C) : 1.4 g/cm^3

Vapour pressure (at 20°C) : 5.5 x 10^{-9} kPa
Water solubility (at 20°C) : 3.1 mg/l
Octanol-water partition coefficient
(log Pow at 20°C) : 3.6

MATERIALS AND METHODS

The materials and methods used for both glasshouse studies and field trials were as described in a previous publication (Godwin *et al.*, 1999). Where appropriate, data were subjected to analysis of variance and treatment separation was achieved via LSD. Treatment means with no letter in common are significantly different at the 5% level.

RESULTS

Broad spectrum control of cereal foliar diseases

On wheat, picoxystrobin provides very good control of *Mycosphaerella graminicola* (*Septoria tritici*), *Phaeosphaeria nodorum* (*Stagonospora nodorum;* syn. *Septoria nodorum*), brown rust (*Puccinia recondita*), tan spot (*Helminthosporium tritici-repentis*) and strobilurin-sensitive powdery mildew (*Blumeria graminis* f.sp. *tritici*). Picoxystrobin provides control of yellow rust (*Puccinia striiformis*) that is sufficient for a moderate disease attack, but mixture with a reduced dose of an effective triazole is necessary to ensure robust control in high risk yellow rust situations. Representative data from European field trials reflecting the relative efficacy of different cereal fungicides (Figures 1 - 3) show how picoxystrobin (250 g ai/ha) compares to current strobilurin-containing commercial products, namely azoxystrobin (250 g ai/ha), kresoxim-methyl/epoxiconazole (125/125 g ai/ha) and trifloxystrobin (250 g ai/ha).

Figure 1. Control of Septoria diseases on wheat

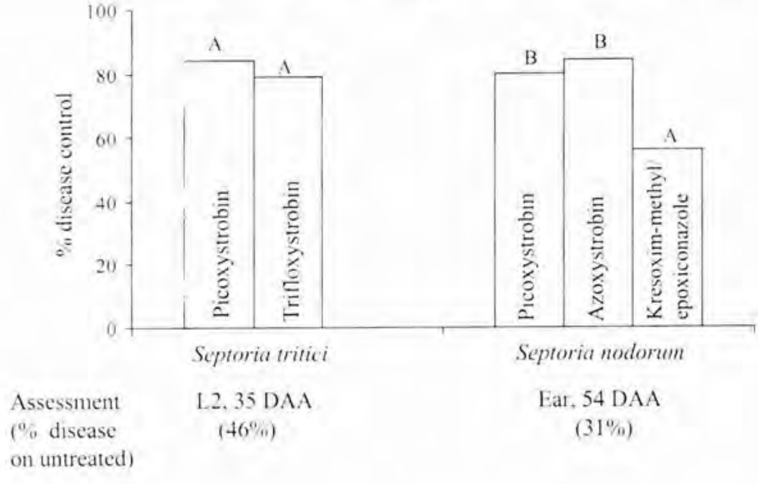
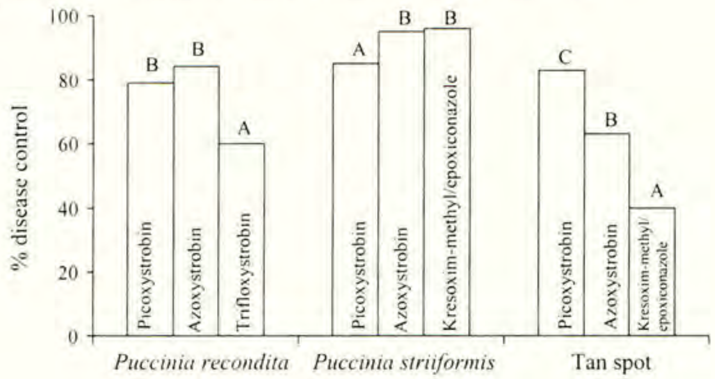


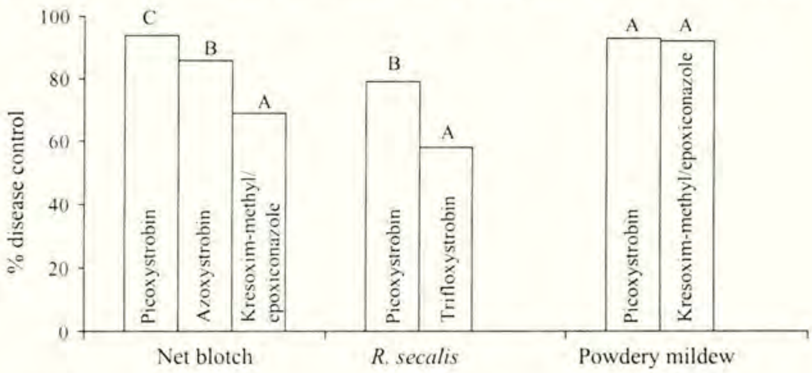
Figure 2. Control of rust diseases and tan spot on wheat



Assessment (% disease on untreated)	L1, 42 DAA (35%)	L1, 30 DAA2 (45%)	L1, 42 DAA (86%)

On barley, picoxystrobin gives very good control of net blotch (*Helminthosporium teres*), *Rhynchosporium secalis*, brown rust (*Puccinia hordei*), *Ramularia* leaf spot (*R. collocygni*) and strobilurin-sensitive powdery mildew (*Blumeria graminis* f.sp. *hordei*). Picoxystrobin also provides broad spectrum disease control on oats (powdery mildew, *Puccinia coronata*, *Helminthosporium avenae*) and rye (powdery mildew, *P. recondita, R. secalis*).

Figure 3. Control of net blotch, *R. secalis* and powdery mildew on barley

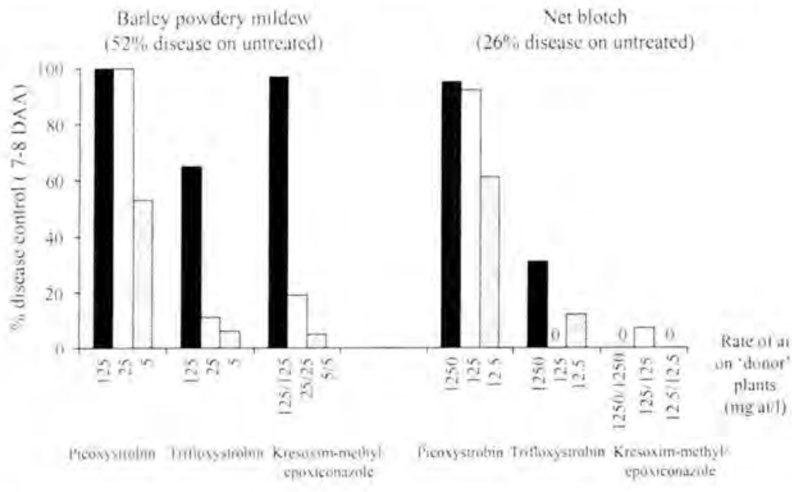


Assessment (% disease on untreated)	L3, 17 DAA2 (71%)	L3, 25 DAA (51%)	L5, 31 DAA (27%)

Redistribution properties

The redistribution properties of picoxystrobin play an important role in delivering its broad spectrum activity against cereal foliar diseases. Picoxystrobin has low volatility, being several orders of magnitude less volatile than fenpropimorph, for example. However, picoxystrobin is intrinsically highly active and therefore the small amount of material that is volatilised can deliver a high level of disease control. By placing untreated seedlings in close proximity to treated 'donor' plants and subsequently inoculating these vapour-treated 'acceptor' seedlings, it has been possible to demonstrate the vapour activity of picoxystrobin against powdery mildew and net blotch of barley in glasshouse studies (Figure 4). Vapour activity is an important property for potent cereal powdery mildewicides in order that they can fully penetrate into the tightly woven 'mycelial mat' on the leaf surface. The vapour activity of picoxystrobin is clearly also of consequence for control of diseases other than powdery mildews.

Figure 4. Vapour activity : disease control on vapour-treated seedlings

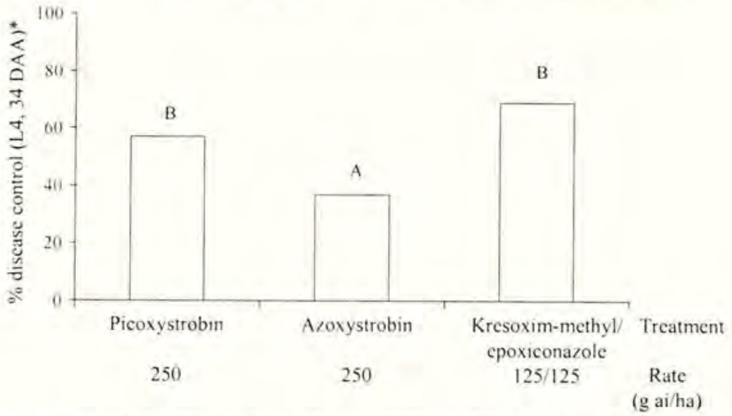


Uptake into the leaf and systemic movement are important properties for the control of deep-seated cereal diseases, e.g. rusts, Septoria diseases and *Helminthosporium* spp. Picoxystrobin shows good uptake, is stable within the leaf and diffuses from the point of absorption to give translaminar disease control. In addition, radiolabel studies and a wheat brown rust bioassay have demonstrated that picoxystrobin is xylem-systemic, showing gradual movement from the zone of application towards the cereal leaf tip. Furthermore, radiochemical studies have shown that picoxystrobin absorbed from the vapour phase into the cereal leaf subsequently demonstrates systemic movement in the xylem. A parallel glasshouse bioassay showed over 80% control of barley brown rust in the leaf tip section following application of picoxystrobin in the vapour phase to a contained basal section of the leaf prior to inoculation.

Curative activity

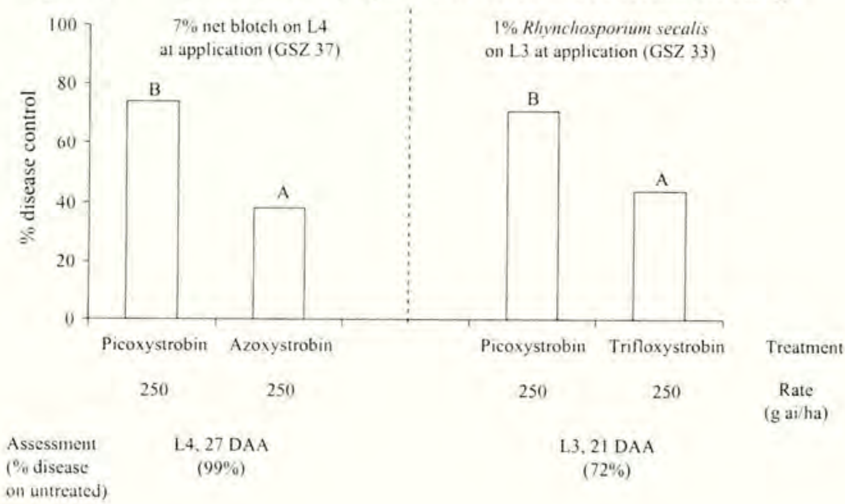
Picoxystrobin has demonstrated greater curative activity than azoxystrobin against *Septoria tritici* in field trials on wheat, although this level of curative activity remains below that of a good triazole (Figure 5). Significant curative activity under field conditions has also been observed with picoxystrobin against net blotch and *Rhynchosporium secalis* on barley (Figure 6). The curative activity of picoxystrobin is explained by its good uptake into the leaf, with around 50% of that applied being taken up into the leaf by 7 days after application. Curative activity is a particularly important feature for cereal fungicides applied early in the season (GSZ 30-33) because disease often lies latent in the crop at this time.

Figure 5. Curative activity against *Septoria tritici* on wheat



* % disease on untreated = 3% on L4 at application (GSZ 33), 57% at assessment

Figure 6. Curative activity against net blotch and *R. secalis* on barley



Yield and quality benefits

Picoxystrobin delivers excellent yield and quality benefits because of its broad spectrum cereal disease control, good maintenance of green leaf area and crop safety. European trials over 3 years against a range of wheat diseases have demonstrated yield benefits with picoxystrobin equivalent to those achieved with kresoxim-methyl/epoxiconazole (Table 1). Only 8 wheat yield trials comparing picoxystrobin to trifloxystrobin were possible because of limited quantities of the commercial formulation of trifloxystrobin being available; *S. tritici*, *P. striiformis* and powdery mildew were the prevalent diseases. The data showed mean yields of 5.9 t/ha for the untreated, 8.8 t/ha for plots treated with a picoxystrobin-based fungicide programme and 8.6 t/ha for plots treated with the equivalent trifloxystrobin-based programme. On barley, picoxystrobin has delivered markedly superior yields to kresoxim-methyl/epoxiconazole. These improvements in wheat and barley yields with picoxystrobin have been accompanied by improvements in grain quality through increases in the frequency of larger-sized grain.

Table 1. Yield responses of wheat and barley

Treatment	Rate (g ai/ha)	Mean yield (t/ha) W. wheat[a] (21 trials)	W. barley[b] (21 trials)
Untreated	-	7.6	6.4
Picoxystrobin	250	9.3	8.1
Kresoxim-methyl/ epoxiconazole	125/125	9.3	7.7

[a] Diseases prevalent: *S. tritici*, *S. nodorum*, *P. recondita*, tan spot, *Cladosporium* spp. and powdery mildew

[b] Diseases prevalent: *R. secalis*, net blotch, *P. hordei* and powdery mildew

Safety and environmental profile

Picoxystrobin, like azoxystrobin, has very good safety and environmental characteristics (Table 2). The mammalian toxicity profile of picoxystrobin indicates that it is benign; it has low acute toxicity, is without reproductive and developmental toxicity and is not genotoxic or carcinogenic. Levels of picoxystrobin residues in cereals are low (<0.01 - 0.20 mg/kg) and it does not accumulate in meat or milk, even following dosing at an exaggerated rate. Calculation of the total daily intake of picoxystrobin using standard European models indicates <5% of the acceptable daily intake is consumed, giving a low human dietary risk. Picoxystrobin demonstrates low toxicity to birds, bees and non-target plants and presents negligible risk to aquatic organisms, earthworms and non-target arthropods under field conditions, and therefore no buffer zones should be required.

CONCLUSIONS

The data presented clearly illustrate that picoxystrobin demonstrates all of the ideal properties for a specialist broad spectrum cereal foliar fungicide.

REFERENCES

Godwin J R; Anthony V M; Clough J M; Godfrey C R A (1992). ICIA5504: a novel, broad spectrum, systemic ß-methoxyacrylate fungicide. *Proceedings of the Brighton Crop Protection Conference - Pests & Diseases*, **1**, 435-442.

Godwin J R; Bartlett D W; Heaney S P (1999). Azoxystrobin: implications of biochemical mode of action, pharmacokinetics and resistance management for spray programmes against Septoria diseases of wheat. In : *Septoria on cereals : a study of pathosystems*, eds J A Lucas, P Bowyer and H M Anderson, 299-315. CABI : Wallingford.

Table 2. Safety and environmental characteristics of picoxystrobin

Mammalian toxicity

End Points	Technical active ingredient	250 g/l SC formulation
Acute MLD's & MLC*	Rat: Oral > 5000 mg/kg; Dermal > 2000 mg/kg; Inhalation > 2.12 mg/l	Rat: Oral > 2000 mg/kg; Dermal > 2000 mg/kg; Inhalation N/A
Skin & Eye irritation	Rabbit: Non-irritating; Non-irritating	Rabbit: Non-irritating; Non-irritating
Skin sensitisation	M&K Guinea Pig: Non-sensitising	Buehler Guinea Pig: Non-sensitising
Dermal absorption	Not applicable (N/A)	Low
Genotoxicity	Non-genotoxic	N/A
Developmental toxicity	Rat & Rabbit: No developmental toxicity potential	N/A
Reproductive toxicity	Rat: No reprotoxicity potential	N/A
Chronic tox./Carcinogenicity	Rat & Mouse: No carcinogenic potential	N/A
Metabolism	Rat: Well absorbed, extensively metabolised, rapidly eliminated	N/A

* MLD = median lethal dose; MLC = median lethal concentration

Environmental fate

Subject	Technical data	Environmental behaviour
Soil persistence	Laboratory aerobic soil DT_{50} values 19-33 days. Field soil dissipation DT_{50} values 3-35 days.	Rapidly degraded in soils with CO_2 as the major product, indicating extensive mineralisation of the molecule. It is not expected to accumulate in soil following normal agricultural use.
Mobility	K_{oc} 790-1200	Not mobile in soil under field conditions.
Aquatic fate	Water phase DT_{50} values 7-15 days from laboratory and outdoor water sediment systems.	Rapid dissipation in water indicates no chronic issues for aquatic organisms.

Environmental toxicity

Non-target group	Hazard information (based on laboratory testing guidelines)	Environmental safety assessed under field use conditions#
Fish	96 h LC_{50} 2 species, 65 - 75 µg/l	Studies on additional species indicate low risk to fish when used under field conditions.
Bioconcentration	Fish whole body BCF 290, low bioconcentration potential.	Unlikely to bioaccumulate in environmental compartments.
Aquatic invertebrates	48 h EC_{50} *Daphnia magna* 18 µg/l	Low risk to aquatic invertebrates. Laboratory and field microcosm studies confirm that the risk to aquatic invertebrates will be low under field conditions.
Aquatic plants	72 h EC_{50} *Selenastrum capricornutum* 56 µg/l	Low risk to aquatic plants. Field microcosm studies confirm the low risk under field conditions.
Sediment-dwelling organisms	28 d *Chironomus riparius* EC_{50} 19 mg/kg (dosed to sediment) 25 d *Chironomus riparius* EC_{50} 140 µg/l (dosed to water)	Low risk to sediment-dwelling organisms either through water or sediment exposure.
Birds	8 d LC_{50} *Colinus virginianus* > 2250 mg/kg 21 week NOEL *Anas platyrhynchos* 1350 mg/kg	Low risk to birds under field conditions.
Bees	48 h LD_{50} > 200 mg/bee	Low risk to bees under field conditions.
Earthworms	14d LC_{50} *Eisenia foetida* 6.7 mg ai/kg soil	Low risk to earthworms under field conditions. A 12 month field trial indicated no adverse effects on earthworm populations under field conditions.
Non-target arthropods	Glass plate 7 d LR_{50} *T. pyri* 12.6 g ai/ha Glass plate 2 d LR_{50} *A. rhopalosiphi* 280 g ai/ha	Laboratory and field tests with 6 species of non-target arthropods under realistic conditions of exposure indicate low risk to populations in both on- and off-crop environments.
Non-target plants	Range of plant species tested in herbicidal screens; no adverse effects seen at the field application rate of 250 g ai/ha.	Low risk to non-target plants under field conditions.
Soil micro-organisms	Applications of 3 times the max. single rate (250 g ai/ha) gave minor transient effects on soil microbial functions. After 28 days these minor differences had disappeared.	Low risk to soil micro-organisms under field conditions.
Overall conclusion is that risk to non-target organisms will be low under field conditions. No buffer zones should be required.		

2 x 250 g ai/ha, 14 day interval

BAS 500 F - the new broad-spectrum strobilurin fungicide

E Ammermann, G Lorenz, K Schelberger
BASF AG, Agricultural Center, 67 114 Limburgerhof, Germany

B Mueller, R Kirstgen, H Sauter
BASF AG, Main Laboratory, 67 056 Ludwigshafen, Germany

ABSTRACT

BAS 500 F is the code number of the new, broad-spectrum strobilurin fungicide developed by BASF. As a foliar spray, it controls the major plant pathogens from the Ascomycete, Basidiomycete, Deuteromycete and Oomycete classes of fungi. BAS 500 F has protectant, curative, translaminar and locosystemic properties, and thus a broad and flexible application window. It is a highly active fungicide for cereals, peanuts and other field crops, grapes, vegetables, bananas, citrus and turf with excellent crop safety. The expected dose rate ranges from 50 - 250 g a.i./ha for food crops and from 280 - 560 g a.i./ha for turf. The compound has a favourable toxicological and ecotoxicological profile and is safe to users and the environment. It is classified by US-EPA as a 'reduced risk candidate'. BAS 500 F is being developed and registered as a solo product and with various pre-mix partners, in a range of formulations. Market introduction is expected for the 2002 season.

INTRODUCTION

BAS 500 F is, after kresoxim-methyl, another result of BASF's intensive synthesis programme initiated following the discovery of synthetic strobilurins as useful tools for the control of plant pathogenic fungi (Müller *et al.*, 1994, Sauter *et al.*, 1999). Since its discovery in 1993, BAS 500 F has been evaluated in an extensive international field test programme over recent years.

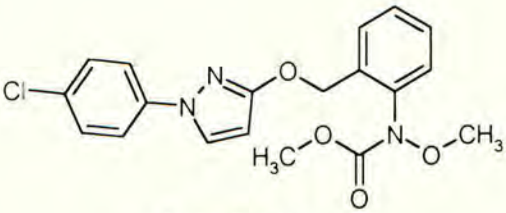
This paper describes its chemical and physical properties, its toxicological and ecotoxicological characteristics, the mode of action, the fungicidal spectrum of activity and its performance in the field.

The trade mark *F 500* is proposed for the active ingredient of BAS 500 F. Its proposed common name is pyraclostrobin.

CHEMICAL AND PHYSICAL PROPERTIES

CAS number:	175013-18-0
Chemical name (IUPAC):	Methyl *N*-(2-{[1-(4-chlorophenyl)-*1H*-pyrazol-3-yl]oxymethyl}phenyl)*N*-methoxy carbamate

Structural formula:



Molecular formula:	$C_{19}H_{18}Cl\ N_3O_4$
Molecular weight:	387.8
Physical state:	white or light beige crystalline solid
Melting point:	63.7 - 65.2°C
Vapour pressure:	2.6×10^{-8} Pa at 20°C
Volatility:	Henry's law constant at 20°C: 5.3×10^{-6} Pa·m³/mol
Partition coefficient n-octanol / water:	log P_{ow}: 3.99 at 22°C
Solubility in water:	1.9 mg / litre at 20°C

PRODUCT SAFETY

Mammalian toxicity:

Acute oral LD_{50}	Rat	> 5000 mg / kg
Acute dermal LD_{50}	Rat	> 2000 mg / kg
Eye irritation	Rabbit	non irritating
Skin irritation	Rabbit	irritant
Skin sensitation (Maximisation)	Guinea Pig	not sensitising
Mutagenicity	5 tests	no mutagenic potential
Teratogenicity	Rat, Rabbit	no teratogenic potential
Chronic toxicity/Carcinogenicity	Rat, Mouse	no carcinogenic potential
Reproduction	Rat	no adverse effects
Metabolism	Rat	rapid absorption and elimination

Toxicity to wildlife:

Bird	Bobwhite quail	LD_{50} > 2000 mg / kg
Fish	Rainbow trout	LC_{50} 0.006 mg / litre
Bees (oral)	not harmful	LD_{50} 310 µg / bee

Environmental fate:

Hydrolysis in water	DT_{50}	> 30 days, stable at pH 5-7 at 25°C
Photolysis in water	DT_{50}	< 2h
Degradation in soil (field studies)	DT_{50}	range 2 - 37 days
Mobility in soil	K_{oc}	6000 - 16000 ml / g

METHODS AND MATERIALS

The field trials were laid out in randomised blocks with 4 replications. The size of the blocks varied from 10 to 200 m². All trials were sprayed at the beginning of attack, either using special small plot tractor spray equipment or a knapsack sprayer. Treatments were applied in 200 - 8000 litres water/ha (8000 l/ha in orange trees). A visual assessment of the % infected leaves, ears or clusters was made for the plot as a whole. Growth stages (GS) are described for mono- and dicotyledonous crops according to BCCH-scale (Anon., 1997).

RESULTS

Mode of action

Biochemically, BAS 500 F acts, like all synthetic strobilurins, as an inhibitor of the bc_1-complex in the mitochondrial respiration. Using a yeast electron transport particle preparation, the rate of ubihydroquinone:cytochrome-c oxidoreductase was inhibited to 50 % by 2.9×10^{-8} mol/l BAS 500 F in comparison with an untreated control. The IC_{50}-value determined for preparations from *Plasmopara viticola* was 2.1×10^{-8} mol/l (F. Röhl, BASF personal communication).

Biological characterisation

BAS 500 F shows very long lasting preventative disease control due to its strong inhibition of spore germination. By its suppression of mycelial development in the leaves, its good curative potential can be explained (Stierl *et al.*, 2000, a and b). Acro- and basipetal trans-port in the leaves and vapour phase activity are very limited but strong translaminar activity has been observed. These characteristics indicate that BAS 500 F is a locosystemic fungicide with very good rainfastness.

Crop safety

BAS 500 F is characterised by excellent crop safety. At the recommended rates, no crop injury has been observed in the vast majority of crops tested. Crop injury may occur in only a few american grape and plum cultivars depending on the growth stage at application.

Field results in cereals

Due to its broad-spectrum fungicidal activity, BAS 500 F is an outstanding tool to control most leaf and ear diseases in cereals and as a result, significant yield increases are achieved. In early curative situations, a single application reduced Septoria blotch (*Septoria tritici*) in wheat very well (Table 1). A similar good control was seen in trials against leaf and glume blotch (*Stagonospora nodorum*) in wheat.

Table 1. Control of *Septoria tritici* on wheat in France, Germany, Poland and UK in 1997 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf area affected	Relative yield[2]
BAS 500 F	250	11	130 c
Azoxystrobin	250	26	123 b
Untreated		54	100 a

[1]Applied at GS 31-43 (mean of 53 trials) [2]Statistically different, P=0.05; Tukey test

Table 2. Control of *Puccinia recondita* and *Drechslera tritici-repentis* on wheat in Germany and UK in 1998 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf area affected and relative yield[2] *P. recondita*		*D. tritici-repentis*	
BAS 500 F	250	2	143 c	3	118 c
Azoxystrobin	250	4	136 b	11	113 b
Untreated		32	100 a	42	100 a

[1]Applied at GS 37-65 [mean of 8 trials (*P. recondita*), 9 trials (*D. tritici-repentis*)]
[2]Statistically different, P=0.05; Tukey test

Rust diseases in cereals, such as brown rust (*Puccinia recondita*) in wheat (Table 2), in barley (*P. hordei*) and stripe rust (*P. striiformis*) in wheat, were effectively reduced by one treatment of BAS 500 F. Tan spot or yellow leaf spot (*Drechslera tritici-repentis*) in wheat (Table 2) as well as net blotch (*Pyrenophora teres*) in barley (Table 3) were also well controlled, even under high disease pressure.

Table 3. Control of *Pyrenophora teres* on winter barley in Germany and UK in 1997 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf area affected	Relative yield[2]
BAS 500 F	250	5	140 c
Azoxystrobin	250	9	135 b
Untreated		40	100 a

[1] Applied at GS 32 - 49 (mean of 21 trials) [2]Statistically different, P=0.05; Tukey test

Due to its broad-spectrum of activity, BAS 500 F has been shown to offer a very good potential for efficient control of other cereal diseases such as spot blotch (*Bipolaris sorokiniana*), snow mould (*Microdochium nivale*), powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat and scald (*Rhynchosporium secalis*) in barley.

Grape vines

BAS 500 F controls powdery mildew (*Uncinula necator*) and downy mildew (*Plasmopara viticola*) on grape vines (Table 4). Under strong disease pressure, both diseases were significantly reduced on leaves as well as on clusters.

Table 4. Control of *Plasmopara viticola* and *Uncinula necator* on grapes in France, Spain and USA in 1997 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf or cluster area affected			
		P. viticola		*U. necator*	
		leaves	clusters	leaves	clusters
BAS 500 F	100	18	4	1	6
Fosetyl-aluminium + folpet	2000 + 1000	28	29	-	-
Quinoxyfen + fenarimol	50 + 15	-	-	1	8
Untreated		74	91	63	85
Number of trials		4		6	

[1]5 - 7 treatments at 12 - 14 day intervals

In addition, BAS 500 F has been shown to have a very good potential to control other important diseases in grape vine, such as black rot (*Guignardia bidwellii*), red fire (*Pseudopezicula tracheiphila*) and dead arm (*Phomopsis viticola*).

Tomatoes and potatoes

In tomatoes and potatoes, BAS 500 F has a very good potential to control the important diseases, early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) (Table 5) and in addition powdery mildew (*Leveillula taurica*) and leaf spot (*Septoria lycopersici*) in tomato.

Table 5. Control of *Phytophthora infestans* on tomatoes in USA in 1997 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf area affected
BAS 500 F	200	20
Chlorothalonil	1125	24
Untreated		86

[1]3-5 treatments at 7 day intervals (mean of 5 trials)

Beans

In beans, BAS 500 F controls the major diseases such as angular leaf spot (*Phaeoisariopsis griseola*), rust (*Uromyces appendiculatus*) and anthracnose (*Colletotrichum lindemuthianum*) on leaves (Table 6) and pods.

Table 6. Control of *Phaeoisariopsis griseola*, *Uromyces appendiculatus* and *Colletotrichum lindemuthianum* on beans in Brazil in 1997 - 2000

Treatment[1]	Dose g a.i. / ha	Mean % leaf area affected *P. griseola*	*U. appendiculatus*	*C. lindemuthianum*
BAS 500 F	75	6	6	2
Azoxystrobin	75	11	9	7
Tebuconazole	150	-	-	14
Untreated		53	51	48
Number of trials		7	4	3

[1]2 - 5 treatments at GS 51 - 73 at 8 - 18 day intervals

Peanuts

BAS 500 F very efficiently controls early leaf spot (*Mycosphaerella arachidis*) and late leaf spot (*Mycosphaerella berkeleyii*) in peanuts (Table 7) as well as leaf blotch (*Phoma arachidicola*), rust (*Puccinia arachidicola*) and scab (*Sphaceloma arachidis*).

Table 7. Control of *Mycosphaerella* spp. on peanuts in Brazil, South Africa and Taiwan in 1998 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf area affected
BAS 500 F	150	15
Tebuconazole	200 - 250	26
Untreated		73

[1]2 - 3 treatments at 21 day intervals (mean of 11 trials)

BAS 500 F also provided good control of southern stem rot (*Sclerotium rolfsii)*.

Citrus

In citrus, the profile of BAS 500 F included good and simultaneous control of the major diseases: scab (*Elsinoe australis* and *E. fawcettii*), melanose (*Diaporthe citri*) and black spot (*Guignardia citricarpa*) (Table 8). In correlation with good disease control, the percentage of marketable fruits was increased.

Table 8. Control of *Elsinoe australis*, *Diaporthe citri* and *Guignardia citricarpa* on orange trees in Argentina, Brazil and South Africa in 1997 - 2000

Treatment[1]	Dose g a.i./1000 litres	Mean % fruits affected and % marketable fruits *E. australis*		*D. citri*		*G. citricarpa*	
BAS 500 F	50	17	97	33	97	22	78
Azoxystrobin	100	32	90	47	81	29	71
Untreated		76	38	71	26	90	10
Number of trials		7		2		3	

[1]2 - 3 treatments at GS 71 - 79 at 34 - 58 day intervals

Turf

In turf, many of the important diseases, such as brown patch (*Rhizoctonia solani*), Pythium blight (*Pythium aphanidermatum*), dollar spot (*Sclerotinia homoeocarpa*), pink snow mould (*Microdochium nivale*) (Table 9), take-all (*Gaeumannomyces graminis*) and leaf spot (*Bipolaris sorokiniana*), were excellently controlled by BAS 500 F.

Table 9. Control of *Rhizoctonia solani, Pythium* spp., *Sclerotinia homoeocarpa* and *Microdochium nivale* on turf in Germany, UK and USA in 1996 - 1999

Treatment[1]	Dose g a.i./ha	Mean % leaf area affected *R. solani*[1]	*P. aphanidermatum*[2]	*S. homoeocarpa*[1]	*M. nivale*[2]
BAS 500 F	250 - 300	4	14	9	5
	500 - 560	-	10	-	-
Azoxystrobin	250 - 300	5	-	-	8
	500 - 560	-	15	-	-
Metalaxyl	750 - 1500	-	18	-	-
Vinclozolin	3000	-	-	7	-
Untreated		62	50	41	25
Number of trials		15	7	8	7

[1]3 - 5 treatments at 14 day intervals , [2]1 - 2 treatments

In many other crops, not mentioned such as banana, coffee, fruits and stone fruits, vegetables, rice, maize, tea, tobacco and ornamentals, BAS 500 F has shown a good potential for efficient control of other important plant diseases.

Resistance

The group of synthetic strobilurins has a proven capacity to select resistant strains of plant pathogenic fungi under field conditions (Anon., 2000). Thus, the use of BAS 500 F will be strictly recommended in tank mixes or in ready formulations with effective fungicides from different chemical groups without cross resistance and with a restricted number of applications per season.

CONCLUSIONS

BAS 500 F is the new broad-spectrum strobilurin fungicide with good protectant, curative, translaminar and locosystemic properties. It is characterised by excellent crop safety. As a foliar spray, BAS 500 F has an excellent potential to control diseases caused by Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes in a broad range of crops. It has a favourable toxicological and ecotoxicological profile and is safe to users and the environment. It is classified by US-EPA as a 'reduced risk candidate'. BAS 500 F is being developed and registered as a solo product and with various pre-mix partners, in a range of formulations.

ACKNOWLEDGEMENTS

The authors would like to thank all colleagues world-wide who have contributed to the development of BAS 500 F.

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A new systemic fungicide - SYP-L190

CH L Liu, W CH Liu, Z CH Li
Shenyang Research Institute of Chemical Industry, Shenyang, P.R.China

ABSTRACT

SYP-L190 is a new systemic fungicide, discovered by Shenyang Research Institute of Chemical Industry in 1994. Tests in the laboratory, greenhouse and field demonstrate that it has very good activity against cucumber downy mildew (*Pseudoperonospora cubensis*), brassica downy mildew (*Peronospora parasitica*), grape downy mildew (*Plasmopora viticola*) and tomato late blight (*Phytophthora infestans*) at 100-200 mg/litre. The primary toxicity results of SYP-L190 show that it has low mammalian toxicity.

The performance characteristics of SYP-L190 are as follows: it is highly active against both metalaxyl-sensitive and resistant isolates of *P. cubensis*; it has good protectant, curative and antisporulant activity; it shows no phytotoxicity to cucumber, cabbage, grape and tomato.

INTRODUCTION

Metalaxyl was the first systemic fungicide to control diseases caused by Oomycetes, though unfortunately, in some pathogens, resistance was soon observed (Georgopoulos & Grigoriu, 1981). The control of plant diseases caused by this group of fungi has become more difficult in recent years with the appearance of phenylamide-resistant pathogens.

SYP-L190, discovered by Shenyang Research Institute of Chemical Industry in 1994, is an Oomycete fungicide acting against members of the families Pythiaceae and Peronosporaceae. It is being developed for markets in which fungicide resistance problems are already occurring. The properties of SYP-L190, as determined in laboratory, greenhouse and field trials, are described below.

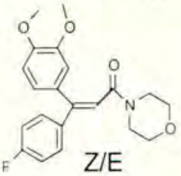
DISCOVERY

To develop new fungicides active against plant diseases caused by Oomycetes, the properties of known products were compared and dimethomorph, derived from a natural product with a novel mode of action and no cross - resistance to known fungicides, was chosen as the lead compound.

Though dimethomorph was reported to have good curative and excellent antisporulant activity (Albert *et al.*, 1988), our test results have shown some differences: the curative effect was about 50% at 150 mg/litre and the inhibition of spore germination was about 45% at 50 mg/litre. Because the introduction of fluorine atoms often imparts desirable physical and biological properties to compounds (Seisaku, 1994; Liu, 1998), a series of fluorine containing analogues of dimethomorph were synthesized (Li *et al.*, 2000). Some of these compounds had much better curative and antisporulant activity than dimethomorph, and one of them, SYP-L190, was chosen to be developed as a fungicide.

CHEMICAL AND PHYSICAL PROPERTIES

Code number:	SYP-L190
Molecular formula:	$C_{21}H_{22}FNO_4$
Molecular weight:	271.4
Appearance at 25ºC:	Colourless crystals
Melting point:	105~110ºC
Structural formula:	(mixture of Z and E isomers, typically Z/E=55:45)



Z/E

Chemical name:	4-[3-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)acryloyl]morpholine
Chemical class:	Cinnamic acid derivative
Solubility:	Easily dissolved in acetone, ethyl acetate, etc.
Stability:	Hydrolytically, photolytically and thermally stable under practical conditions (20-40°C)
Formulation:	10% EC and 20% WP; other formulations are under study.

TOXICOLOGY

Acute toxicity:	Oral LD_{50} (rat)	>2710 mg/kg (male), >3160 g/kg (female)
	Dermal LD_{50} (rat)	>2150 mg/kg (male), >2150 mg/kg (female)
Irritation:	Eye (rabbit)	Non-irritating
	Dermal(r abbit)	Non-irritating

No mutagenic, teratogenic or carcinogenic effects were observed (Ames test, micronucleus tests).

BIOLOGICAL ACTIVITY

Inhibition of spore germination of cucumber downy mildew

Activity against spore germination of *Pseudoperonospora cubensis* was tested in the laboratory. SYP-L190 (10% EC) at 100, 50 and 25 mg/litre was compared with dimethomorph (commercially available product, 50% WP) at 50 mg/litre and an untreated control. A spore suspension was prepared from cucumber leaves affected by downy mildew. This was mixed with the solution of the test compound, and each treatment was tested 6 times. Treated samples were kept in a constant temperature incubator (25°C), and examined after 24 hours. Eighteen microscopic fields were examined for each treatment, and the numbers of germinated and ungerminated spores were counted. SYP-L190 was highly effective in inhibiting spore germination, and its inhibitory action was superior to dimethomorph (Table 1).

Table 1. Inhibition of spore germination of *Pseudoperonospora cubensis* by SYP-L190

Fungicide	Concentration (mg/litre)	% spore germination	% inhibition of spore germination
SYP-L190	25	23.1	65.2
	50	4.3	93.5
	100	1.6	97.6
Dimethomorph	50	38.2	42.5
Untreated	-	66.4	-

Phytotoxicity to cucumber

The concentration of SYP-L190 was set at 500, 800, 1000, 2000 and 4000 mg/litre, and each treatment was tested three times. Tests were conducted on seedlings of yellow melon at the 2-3 leaf stage. Results are shown in Table 2. SYP - L190 did not show any phytoxicity to cucumber at concentrations up to 1000 mg/litre.

Table 2. Test of phytotoxicity to cucumber

Chemical	Concentration	Days after treatment						
	(mg/litre)	1	3	5	7	9	12	14
SYP - L190	500	-	-	-	-	-	-	-
	800	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-
	2000	-	+	+	+	+	+	+
	4000	-	+++	+++	+++	+++	+++	+++
Dimethomorph	2000	-	-	-	+	+	+	+
Untreated	-	-	-	-	-	-	-	-

Note: - no phytoxicity, + slightly small or yellow leaves, +++ growth stopped.

Protectant and curative activity against cucumber downy mildew

A comparison of protective and curative activity of SYP-L190 (10% EC) against cucumber downy mildew was made in a further trial. The concentrations of SYP-L190 and dimethomorph were set at 150 and 100 mg/litre, and each treatment was tested three times. Tests were conducted on seedlings of yellow melon at the 2-3 leaf stage. For the protectant test, fungicide treatment was applied 24 hours before inoculation. For the curative test, SYP-L190 was applied 24 hours after inoculation. Plants were examined a week later, categorised into 9 grades, and a disease index (DI) on a 0 to 1.0 scale was calculated (a disease index of 1.0 represents all plants severely affected). Results are shown in Table 3. Both curative and protective effects of SYP-L190 were better than that of dimethomorph. SYP-L190 showed better activity when used as a protectant treatment than when used as a curative treatment.

Table 3. Protectant and curative activities of SYP-L190 and dimethomorph against *Pseudoperonospora cubensis*

Fungicide	Concentration (mg/litre)	Protectant test		Curative test	
		DI	% control	DI	% control
SYP-L190	100	0.07	93	0.37	63
	150	0.05	95	0.26	74
Dimethomorph	100	0.39	61	0.98	2
	150	0.21	79	0.76	24
Untreated	-	1.00	-	1.00	-

Some other currently available fungicides were compared with SYP-L190 using the same test procedure (Table 4). The curative and protective effects of SYP-L190 at 100 mg/litre were equivalent to chlorothalonil (1000 mg/litre), but the fungicide was more effective than metalaxyl, aluminium phosphate, mancozeb and propamocarb hydrochloride when used at 200 mg/litre as a protectant.

Table 4. A comparison of the protectant and curative activities of SYP-L190 and other fungicides against *Pseudoperonospora cubensis*

Fungicide	Concentration (mg/litre)	Protective effect (% control)	Curative effect (% control)
SYP-L190	25	40	0
	50	60	40
	75	75	60
	100	90	75
	200	100	90
Metalaxyl	500	60	40
Aluminum phosphate	1000	75	40
Chlorothalonil	1000	90	75
Mancozeb	1000	74	0
Propamocarb hydrochloride	1000	90	0
Untreated		0	0

The activity of SYP-L190 against metalaxyl-resistant strains of cucumber downy mildew was tested using the same procedure (Table 5). SYP-L190 was highly active against metalaxyl-resistant strains of the fungus. There was no cross-resistance between SYP-L190 and metalaxyl.

Table 5. Activity of SYP-L190 against metalaxyl-resistant strains of cucumber downy mildew

Fungicide	Concentration (mg/litre)	Disease index	Control (%)
SYP-L190	100	0.09	91
	200	0	100
Metalaxyl	500	1.0	0
	1000	1.0	0
Chlorothalonil	1000	0.42	58
Untreated	-	1.0	-

Field protective test against cucumber downy mildew

All field trials were conducted using plots of 20 - 50 m^2 and with 3 or 4 replicates of each treatment. This protectant experiment was carried out on a farm near Shenyang City. SYP-L190 (20% WP) was tested at 200 and 600 mg/litre in comparison with propamocarb hydrochloride (66.5% hydrate) at 800, 1000 and 1200 mg/litre. Each treatment was applied three times, on 5, 17 and 22 April. At the second treatment on April 17, spread of infection was seen. Assessment of the protective effect was made on April 22, April 29 and May 5.

Table 6. Protective effect of SYP-L190 against cucumber downy mildew in the field

Fungicide	Concentration	April 22		April 29		May 5	
	(mg/litre)	DI	% control	DI	% control	DI	% control
SYP-L190	200	0	100	0	100	0.03	97.0
	400	0	100	0	100	0	100
	600	0	100	0	100	0	100
Propamocarb	800	0.09	89.2	0.21	77.2	0.59	41.0
hydrochloride	1000	0.06	91.9	0.16	82.6	0.42	58.0
	1200	0.03	95.9	0.10	89.1	0.32	68.0
Untreated	-	0.74	-	0.92	-	1.00	-

SYP-L190 at 200, 400 and 600 mg/litre showed a far better protective activity than propamocarb hydrochloride at 800 - 1200 mg/litre. Fourteen days after the third spray, SYP-L190 at all three concentrations was more effective than propamocarb hydrochloride.

Field curative tests against cucumber downy mildew

This test was also carried out on a farm near Shenyang City. Concentration of SYP - L190 (20% WP) was 100, 200 and 300 mg/litre. Cymoxanil + mancozeb (72%WP, mixture of 8% cymoxanil and 64% mancozeb) at 1500 mg/litre was used as the reference treatment, and an untreated control was also used. Each treatment was applied three times, on 13, 20 and 27 May. The disease levels were assessed on 3 June. Results are shown in Table 7.

Table 7. Curative effect of SYP - L190 against cucumber downy mildew (Experiment 1)

Chemicals	Concentration (mg/litre)	DI	% control
SYP-L190	100	0.049	88.4
	200	0.011	97.7
	300	0	100.0
Cymoxanil + mancozeb	1500	0.076	81.9
Untreated	-	0.422	-

The curative effect of the SYP - L190 at 100 mg/litre was slightly better than cymoxanil + mancozeb at 1500 mg/litre (Table 7).

In a second experiment, SYP-L190 (20% WP) was tested at 160 mg/litre against propamocarb hydrochloride at 667 mg/litre. Each treatment was repeated three times. Most of the leaves had got disease on April 28 before the first spray was applied. Treatments were applied on April 29 and May 6 and the disease assessed on May 14 (Table 8). SYP-L190 gave good control of cucumber downy mildew, even under 80% disease pressure. Control was better than that from propamocarb hydrochloride.

Table 8. Curative effect of SYP-L190 against cucumber downy mildew (Experiment 2)

Fungicide	Concentration (mg/litre)	April 28 Diseased leaves (%)	May 14 Diseased leaves (%)	% control
SYP-L190	160	81.4	21.6	75.5
Propamocarb	667	79.8	57.8	33.2
Untreated	-	83.3	90.3	-

In a third experiment against cucumber downy mildew, SYP-L190 (20% WP) was tested at 160 mg/litre against cymoxanil + mancozeb (72%) at 1200 mg/litre. The disease was observed on 5 April, and treatments were applied on 8 and 16 April. The crop was assessed 10 days later. The efficacy of SYP-L190 at 160 mg/litre was much better than cymoxanil + mancozeb at 1200 mg/litre (Table 9).

Table 9. Curative effect of SYP - L190 against cucumber downy mildew (Experiment 3)

Chemical	Concentration	April 7 (before treatment)		April 26		
	(mg/litre)	Disease leaves (%)	Disease index	Disease leaves (%)	Disease index	% control
SYP - L190	160	28.6	0.40	13.2	0.09	91.3
Cymoxanil + Mancozeb	1200	24.4	0.32	14.5	0.16	80.6
Untreated	-	26.8	0.26	55.7	0.67	-

Field curative test against brassica downy mildew

Activity against brassica downy mildew (*Peronospora parasitica*) was evaluated on cabbage in a field trial. SYP-L190 (20% WP) was tested at 200 mg/litre and cymoxanil + mancozeb (72%) at 1200 mg/litre. Each treatment was applied twice, on 20 and 29 August. Disease levels were assessed on 2 and 27 September and a disease index was calculated. Results are shown in Table 10. The efficacy of SYP-L190 at 200 mg/litre was much better than cymoxanil + mancozeb at 1200 mg/litre.

Table 10. Curative effect of SYP - L190 against brassica downy mildew

Chemical	Concentration (mg/litre)	September 2		September 16	
		DI	% control	DI	% control
SYP - L190	200	0.84	94.3	2.63	92.8
Cymoxanil + mancozeb	1200	4.28	70.8	8.41	77.1
Untreated	-	14.64	-	36.75	-

Field test against tomato late blight

Activity against tomato late blight (*Phytophthora infestans*) was evaluated in a field trial. SYP-L190 (20% WP) was tested at 200, 400 and 600 mg/litre and mancozeb (80% WP) at 1300 mg/litre. Each treatment was applied three times, on May 21, May 28, and June 5. % leaf area affected by blight was assessed on June 20 and a disease index was calculated. SYP-L190 at 400 and 600 mg/litre was more effective against tomato late blight than mancozeb at 1300 mg/litre (Table 11).

Table 11. Effect of SYP-L190 against tomato late blight in the field

Fungicide	Concentration (mg/litre)	DI before treatment	DI after treatment	% control
SYP-L190	200	0.74	5.56	80.9
	400	0.74	2.59	91.1
	600	1.11	2.22	94.9
Mancozeb	1300	1.11	5.56	87.3
Untreated	-	1.11	43.70	-

Field protective test against grape downy mildew

Activity against grape downy mildew (*Plasmorpara viticola*) was evaluated in a field trial. SYP-L190 (20% WP) was tested at 67, 100 and 200 mg/litre and azoxystrobin (25% WDG) at 100 mg/litre. Each treatment was applied three times, on July 29, August 11 and August 18. The % leaf area affected was assessed on August 25 and a disease index was calculated. SYP-L190 at 100 mg/litre showed similar protective effect to azoxystrobin at 100 mg/litre (Table 12).

Table 12. Protective effect of SYP-L190 against grape downy mildew in the field

Chemical	Concentration mg/litre	DI	% control
SYP-L190	67	7.03	76.93
	100	4.17	86.97
	200	4.14	87.52
Azoxystrobin	100	5.81	81.39
Untreated	-	32.3	-

DISCUSSION

Tests over five years in the laboratory, the greenhouse and in field trials, demonstrate that SYP-L190 has good activity against cucumber downy mildew, brassica downy mildew, grape downy mildew and tomato late blight at 100-200 mg/litre. The results were superior to those of the existing standards tested. The activity of SYP-L190 embraces residual, protectant, curative and antisporulant components. No phytotoxicity or other adverse effects on crop growth have been observed. No cross-resistance to phenylamides has been observed. SYP-L190 will provide a valuable addition to the current range of Oomycete fungicides.

The mode of action of SYP-L190 is currently under study. Further evaluation of SYP-L190 against tomato late blight and grape downy mildew, and tests on other diseases, are in progress.

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Simeconazole (F-155), a novel systemic fungicide with broad-spectrum activity for seed treatment

M Tsuda, H Itoh, K Wakabayashi, T Ohkouchi, S Kato
Agroscience Research Laboratories, Sankyo Co., Ltd., Yasu, Shiga, 520-2353, Japan

K Masuda, M Sasaki
Research Laboratory Hokkai Sankyo Co., Ltd., Kitahiroshima, Hokkaido, 061-1111, Japan

ABSTRACT

Simeconazole, 2-(4-fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-trimethylsilylpropan-2-ol, is a novel triazole fungicide with prominent systemic effects and good crop safety. It shows broad and strong antifungal activity against plant pathogens, especially those of the Basidiomycetes. Seed treatment with simeconazole achieves excellent efficacies against wheat loose smut (*Ustilago nuda*) at doses of 4-10 g a.i./100 kg seed. At high doses of 50-100 g a.i./100 kg seed, the controlled-release formulation of simeconazole is also effective against soil and airborne diseases such as sharp eyespot (*Rhizoctonia cerealis*), eyespot (*Pseudocercosporella herpotrichoides*), and powdery mildew (*Blumeria graminis*). Simeconazole increases wheat yield by approximately 10% over untreated control crops.

INTRODUCTION

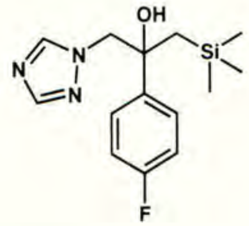
Simeconazole is now being introduced by Sankyo as the first triazole fungicide for the control of rice sheath blight caused by *Thanatephorus cucumeris*, using a submerged application in paddy water. Simeconazole has a broad spectrum of activity and the characteristics of prominent systemic movement and crop safety. Due to its broad spectrum of activity, simeconazole can also control other sclerotial diseases on rice caused by *Ceratobasidium setariae*, *Waitea circinata*, *Thanatephorus cucumeris* (*Rhizoctonia solani* AG2-2), and *Sclerotium fumigatum*.

Simeconazole preparations are also under development for disease control in fruits, vegetables, and turfgrass. Among target diseases, simeconazole shows excellent control efficacy against scab (*Venturia inaequalis*), blossom blight (*Monilinia mali*), rust (*Gymnosporangium yamadae*), and powdery mildew (*Podosphaera leucotricha*) on apple trees, and large patch (*Rhizoctonia solani* AG2-2LP), brown patch (*Rhizoctonia solani*, AG2-2), and dollar spot (*Sclerotinia homoeocarpa*) on turfgrass.

This paper describes its chemical properties and the novel fungicidal profiles as a seed treatment fungicide on cereals.

CHEMICAL AND PHYSICAL PROPERTIES

Code number:	F-155
Chemical name (IUPAC):	2-(4-fluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-trimethylsilylpropan-2-ol
Common name (BSI/ISO):	Simeconazole
Structural formula:	



Molecular formula:	$C_{14}H_{20}FN_3OSi$
Molecular weight:	293.4
Appearance at 20°C:	White crystals
Melting point:	118.5-120.5°C
Vapor pressure:	5.4×10^{-5} Pa at 25°C
Solubility:	57.5 mg/litre water at 20°C
Partition coefficient:	Log P = 3.2 (n-octanol / water)

TOXICOLOGY

Acute oral LD_{50}	(rat, males):	611 mg/kg body weight
	(rat, females):	682 mg/kg body weight
Acute dermal LD_{50}	(rat, males and females):	>5000 mg/kg body weight
Skin irritation (rabbit):	Non-irritating	
Eye irritation (rabbit):	Non-irritating	
Mutagenicity:	Non-mutagenic in Ames test	
Teratogenicity:	Not a teratogen in rat and rabbit	

MATERIALS AND METHODS

Simeconazole was synthesized (Itoh *et al*., 2000) and formulated as 20% wettable powders with non-controlled and controlled-release formulations by the Agroscience Research Laboratories, Sankyo Co., Ltd. Each formulation was thoroughly mixed with seeds by hand followed by Dhingra's method (Dhingra & Sinclair, 1995).

Field trials were carried out in Shiga in central Japan (experimental station of the Agroscience Research Laboratories, Sankyo) and Hokkaido in northern Japan (experimental station of the Research Laboratory, Hokkai Sankyo). Treated seeds were drilled at a rate of

150 kg seeds /ha. Wheat loose smut (*Ustilago nuda*) trials were conducted in 1997-1998 in Shiga and in 1998-1999 in Hokkaido. The uncontrolled-release formulation 20% WP of simeconazole was evaluated. We used wheat seeds that were collected from naturally infected ears in the previous year.

Powdery mildew (*Blumeria graminis*), eyespot (*Pseudocercosporella herpotrichoides*), and sharp eyespot (*Rhizoctonia cerealis*) trials were conducted in Hokkaido in 1993. The controlled-release formulation was evaluated and compared with the uncontrolled-release one. Eyespot and sharp eyespot were artificially inoculated in the soil of the test field before sowings. Assessments were made at growth stage (GS) 30-45 and GS 75 according to established standard methods (Matthews *et al* ., 1985).

The crop safety of simeconazole seed treatment was examined in a greenhouse pot test. The seed germination rate and plant height were assessed. The effect on the yield of the crop was also assessed in a field trial in 1993-1994 in Shiga without artificial inoculation of any pathogen.

RESULTS

Antifungal activity

The antifungal activity of simeconazole is shown in Table 1. The chemical strongly inhibited mycelial growth of plant pathogens especially of Basidiomycete fungi such as *Ustilago maydis*, *Thanatephorus cucumeris*, and *Ceratobasidium gramineum*.

Table 1. *In vitro* activity of simeconazole against various fungi on potato dextrose agar plates (IC_{50} values a.i. in mg/litre).

Crop	Disease	Pathogen	IC_{50}
Wheat	Eyespot	*Pseudocercosporella herpotrichoides*	0.26
	Take-all	*Gaeumannomyces graminis*	0.56
	Fusarium blight	*Gibberella zeae*	2.60
	Fusarium blight	*Fusarium culmorum*	2.75
Barley	Foot-rot	*Ceratobasidium gramineum*	0.05
	Stripe	*Pyrenophora graminea*	0.78
Maize	Leaf spot	*Helminthosporium maydis*	0.27
	Smut	*Ustilago maydis*	0.16
Apple	Scab	*Venturia inaequalis*	0.25
	Blossom blight	*Monilinia mali*	<0.3
Strawberry	Southern blight	*Sclerotium rolfsii*	0.53
	Verticillium wilt	*Verticillium dahliae*	0.41
Rice	Sheath blight	*Thanatephorus cucumeris*	0.03
	Brown sclerotium disease	*Ceratobasidium setariae*	0.36

Loose smut control in the field

In Shiga, the efficacy of simeconazole against wheat loose smut was excellent at 4-15 g a.i./100 kg seed, which was the same dose range used for the reference fungicide triadimenol (Table 2). The trial in Hokkaido also demonstrated that simeconazole controls wheat loose smut (Table 3). No delay in germination and seedling growth at the dose of 4-15 g a.i./100 kg seed was observed (data not shown). Furthermore, we confirmed the effect of simeconazole against loose smut in barley and bunt in wheat in Germany.

Table 2. Efficacy of simeconazole against loose smut on winter wheat in a field trial (Shiga, 1997-1998).

Chemical	Dose rate (g a.i./100 kg seed)	Number of infected (ears/m^2)	Disease control (%)
Simeconazole	15	0.1	99.7
	10	0.1	99.7
	8	0.9	97.3
	6	0.4	98.8
	4	0.3	99.1
Triadimenol	15	0.2	99.4
	10	0.1	99.7
	8	0.8	97.6
	6	1.3	96.1
	4	0.8	97.6
Triflumizole	150	3.1	90.8
Untreated	-	33.7	-

Table 3. Efficacy of simeconazole against loose smut on winter wheat in a field trial (Hokkaido, 1998-1999).

Chemical	Dose rate (g.i./100 kg seed)	Number of infected (ears/m^2)	Disease control (%)
Simeconazole	30	0.0	100.0
	24	0.0	100.0
	18	0.4	95.0
	10	0.2	97.5
Iminoctadine + benomyl	125+100	0.6	92.5
Untreated	-	8.0	-

Effect of controlled-release formulations

The controlled-release formulation of simeconazole at high doses of 50-100 g a.i./100 kg seed, provided a long duration of protection against sharp eyespot, eyespot, and powdery mildew (Figure 1). The uncontrolled-release formulation provided an excellent efficacy against sharp eyespot but poor efficacies against eyespot and powdery mildew.

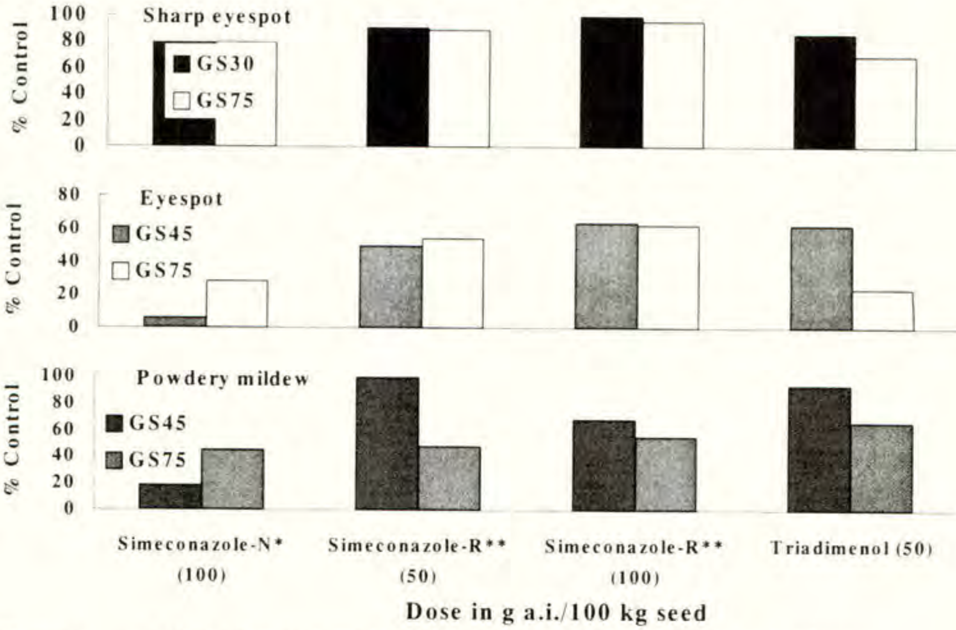


Figure 1. Effect of simeconazole seed treatment against soil borne and airborne diseases on winter wheat (Japan, 1993-1994).
* : Uncontrolled-release formulation of simeconazole WP
**: Controlled-release formulation of simeconazole WP

Effect of seed treatment with simeconazole on plant growth and yield

The effect of simeconazole on wheat yield was evaluated in a field test (Figure 2). Seed treatment with simeconazole at a dose of 50-100 g a.i./100 kg seed increased the wheat yield from 3.84 t/ha (untreated control) to 4.29-4.35 t/ha. This corresponded to a 10% relative improvement in yield. On the other hand, yield benefits from triadimenol treatment were not observed in this trial, though triadimenol was as effective against powdery mildew and eyespot as simeconazole.

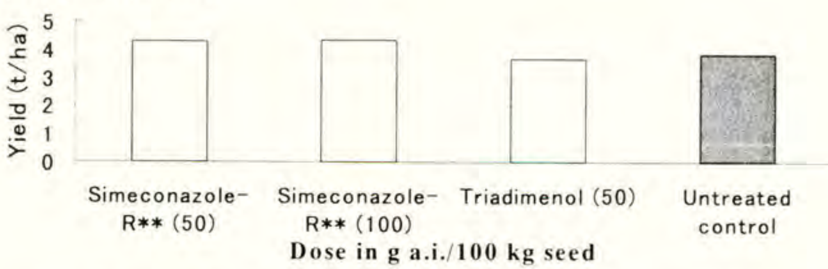


Figure 2. Effect of simeconazole seed treatment on winter wheat yield (Japan, 1993-1994)
*,**: As shown in Figure1.

DISCUSSION AND CONCLUSIONS

The effectiveness of simeconazole against loose smut of wheat is confirmed in field trials. Simeconazole seed treatment at a dose of 4-10 g a.i./100 kg seed achieves an average control of 97%, exceeding the minimum 95% requirement for registration in Germany by 2%. Seed treatment of simeconazole is effective against not only seedborne diseases but also soil and airborne diseases on cereals such as smut, eyespot, sharp eyespot, and powdery mildew. Due to its systemic movement, simeconazole shows excellent control efficacy against deep-seated seed-borne diseases such as loose smut and some soil and airborne diseases on wheat. Indeed, because of the very fast uptake of simeconazole by rice plants, the chemical can be detected in plants three days after the submerged application and provides excellent efficacy against sheath blight.

A significant increase in wheat yield, of approximately 10% over the untreated control, is obtained by seed treatment with simeconazole. We postulate that the increased yield resulted from the near complete suppression of disease which reduces the quantity and quality of yield by the simeconazole seed treatment. In rice plants, we confirm that simeconazole was successful for the control of ear diseases such as ear blight (*Cochliobolus miyabeanus*) and false smut (*Claviceps virens*) by a submerged application in paddy fields.

Thus, simeconazole is a useful tool to control not only bunts and smuts, but also other soil-borne and airborne diseases through the use of controlled-release formulations. Moreover, it can reduce the number of fungicides applied to cereals.

ACKNOWLEDGEMENTS

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Brevibacillus brevis – a novel candidate biocontrol agent with broad-spectrum antifungal activity

B Seddon, R C McHugh
Department of Agriculture & Forestry, MacRobert Building, University of Aberdeen, Aberdeen, AB24 5UA, Scotland, UK

A Schmitt
Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Heinrichstr.243, 64287 Darmstadt, Germany.

ABSTRACT

Brevibacillus brevis (formerly *Bacillus brevis*) inhibits a range of fungal plant pathogens *in vitro* including *Botrytis cinerea*, *Sphaerotheca fuliginea* and *Pythium ultimum*. Generally biological control agents (BCAs) operate in niche situations whereas this wide range of sensitive pathogens should allow the development of *B. brevis* as a BCA. *B. brevis* has two modes of antagonism: the antifungal metabolite, gramicidin S, and a biosurfactant that reduces periods of surface wetness. A range of pathogens and crop disease situations have been investigated and efficacy demonstrated against aerial plant pathogens in greenhouse crops (lettuce, tomatoes and cucumbers). When wetness predominates, gramicidin S appears to be the mode of antagonism against *B. cinerea* whereas during interrupted periods of wetness the biosurfactant plays a more substantial role. Constraints on the success of the applications are identified and strategies suggested to overcome these including integrated control with other biological methods and fungicides. *B. brevis* holds promise as a broad-spectrum BCA with more than one mode of action as an alternative to chemical control.

INTRODUCTION

Concerns about the environment and the inevitable appearance of fungicide-resistant strains mean that alternatives to fungicides are necessary for disease control. These methods need to be environment friendly and give sustainable crop protection. The aim is to reduce the use of chemical fungicides to the minimal level possible or to alternate or integrate their use with cultural methods and the use of biological control agents (BCAs) (Seddon *et al.*, 1997). Most BCAs active against fungal plant pathogens operate through antagonism whether this be antibiosis, parasitism, competition for nutrients and space or other means. With many antagonists this is a rather specific interaction with one pathogen targeted in a single crop. The result is that the use of BCAs to control diseases is restricted to niche situations. The main benefit of chemical fungicides is their use against a wide range of fungi in diverse crop situations; their development therefore by agrochemical companies is financially viable. BCAs are currently limited to small niche markets and play only a minor role in crop protection. However, it should be financially worthwhile to develop and exploit a BCA active against a range of fungal plant pathogens and with potential for use in many diverse cropping systems.

BREVIBACILLUS BREVIS AND BIOCONTROL

Bacilli have a wide-spectrum antibiosis activity against many microorganisms and their spores are able to withstand harsh environmental conditions. They are ideal candidates therefore for development as BCAs both for formulation and activity (Rhodes, 1990). For several years *Bacillus brevis* (now renamed *Brevibacillus brevis*) (Shido *et al.*, 1996) has been studied in our laboratory with the aim of developing it as a BCA against a range of fungal plant pathogens. *Botrytis cinerea* is an important plant pathogen of many crops and was initially the target of our studies. Biocontrol was first demonstrated with *B. cinerea* on Chinese cabbage (Edwards *et al.*, 1994). More recently, pathogenic *Pythium* spp. (Walker *et al.*, 1998) and also cucumber powdery mildew (*Sphaerotheca fuliginea*) (Seddon & Schmitt, 1999), have been studied. *In vitro* experiments indicate that *Phytophthora infestans*, *Gaeumannomyces graminis*, *Pyrenophora graminea*, *Rhizoctonia solani*, and *Microdochium nivale* are all antagonised by *B. brevis* and gramicidin S (Murray *et al.*, 1986; Edwards *et al.*, 1994).

MODES OF ANTAGONISM

B. brevis strain Nagano produces the cyclic decapeptide gramicidin S (Edwards *et al.*, 1994). Both conidial germination and mycelial growth are inhibited in *Botrytis cinerea* but the level of inhibition is influenced by the composition and state of the medium. Greater sensitivity to gramicidin S can be demonstrated by conidia when germinating in liquid media under shake conditions than when the liquid is static, and this in turn is greater than when germination occurs on the surface of a leaf (Chinese cabbage). These parameters may reflect different situations in liquid droplets and on the surface of crop plants in the greenhouse. Inhibition of mycelial growth also shows similar sensitivity differences. However, mycelial growth is much less sensitive to inhibition by gramicidin S (50-500 μM) than is conidial germination (5-15 μM) (Edwards, 1993). For this reason, conidial germination is targeted as the developmental stage of *B. cinerea* most likely to be antagonised by *B. brevis*. Conidia are also the propagules that initiate disease and that continue the polycyclic nature of grey mould epidemics.

Gramicidin S is known to interact with biological membranes (Seddon *et al.*, 1996). Possibly germinating conidia and germ tubes are more susceptible to inhibition than mycelia simply because the cytoplasmic membrane is more exposed due to the limited deposition of cell-wall material at this developmental stage. The composition of the cytoplasmic membrane may also differ here to its composition during mycelial growth and this could lead to greater sensitivity to gramicidin S. Gramicidin S is known to interact with phospholipid moieties of membranes and the exposure of these within the structure of the membrane may change depending on the membrane composition. On treatment of conidia with gramicidin S there is a rapid loss of cellular ATP which is consistent with rapid and irreversible loss of membrane integrity. A gramicidin S-deficient mutant (E-1) can be used in comparative studies with the wild type (WT) to investigate the effects of gramicidin S produced *in situ* (Edwards *et al.*, 1994). Gramicidin S produced by *B. brevis* is associated with the outer surface of the bacterial spore and spores of *B. brevis* WT are equally as effective as the isolated and purified gramicidin S in inhibiting conidial germination of *B. cinerea*. *B. brevis* E-1 does not produce gramicidin S and these spores do not inhibit conidial germination. These studies demonstrated unequivocally that it is the gramicidin S carried by the spores of *B. brevis* WT that is the means of antagonism in these *in vitro* studies (Edwards, 1993).

B. brevis also produces a biosurfactant which leads to reduced periods of surface wetness thereby preventing conidia completing germination on plant surfaces and establishing disease (Seddon *et al.*, 1997). This mode of action is not demonstrated with *in vitro* studies in solution nor on the surface of agar and leaf discs at 100% RH since surface evaporation is not possible under these conditions. In situations where RH is not 100% and there is loss of water from plant surfaces, the biosurfactant activity has been demonstrated to reduce periods of surface wetness four-fold (Seddon *et al.*, 1997). Both *B. brevis* WT and E-1 produce biosurfactant and so, where the mode of antagonism is via reduced periods of surface wetness, the two strains should be equally effective in controlling disease. In crop situations where RH is high it would be expected that only *B. brevis* WT would be effective since the biosurfactant will not be operative in reducing periods of surface wetness and any disease control here would be the result of the gramicidin S. Using both strains of *B. brevis* WT and E-1 in field tests and in different crop environments it should be possible to demonstrate whether gramicidin S or biosurfactant or both are operative in disease control.

DISEASE CONTROL

Grey mould

Tomato crop

Grey mould in tomato crops grown in polythene greenhouses in Crete was demonstrated to be controlled by both *B. brevis* WT and E-1. Disease control on leaves was about 40% and there was no significant difference between the disease control levels of *B. brevis* WT and E-1 (Seddon *et al.*, 1997). The tomato crop in Crete is grown over the winter period (September-May) and grey mould begins around January and can reach epidemic proportions by April. It is during this period that day and night time temperatures can fluctuate considerably resulting in longer periods of high humidity which promote germination of *Botrytis* conidia. Since control by *B. brevis* WT and E-1 was identical, these observations are consistent with the suggestion that periods of leaf wetness are reduced sufficiently by the biosurfactant on the *B. brevis* treated leaves to prevent conidia completing the germination sequence and infecting leaves.

In Scotland, similar tomato trials were carried out in unheated polythene tunnels during the summer growing season when again fluctuating day and night temperatures can lead to relatively long periods of overnight surface wetness. Treatment was compared to iprodione (Rovral WP at 1g/l). Control of *Botrytis* leaf infection with *B. brevis* (7 sprays at 14 d intervals from 13 d after planting) was about 40-45% with no significant difference between WT and E-1 treatments indicating again that biosurfactant activity is the major component in suppressing the disease (Table 1). However when stem infection was monitored there was a significant difference between protection afforded by *B. brevis* WT and E-1. Stem lesions caused by *B. cinerea* occurred on most (80-90%) untreated plants. *B. brevis* WT reduced this to about 30% of plants having stem lesions and the lesions which did occur on WT treated plants were less severe. *B. brevis* E-1 only reduced stem lesions to a level of about 60%

(Table 1) and these lesions were more severe than treatment with WT. In this situation therefore, gramicidin S seems to be playing a more significant role in disease control than the biosurfactant. The surface area to volume ratio of the leaf is much greater than that of the stem and the biosurfactant would be expected to make a greater contribution to the drying of leaves than of stems. Also guttation from stem wounds would maintain wetness for longer. It had already been shown that gramicidin S can interact with components of the leaf surface reducing its activity against *B. cinerea* (Edwards, 1993). Presumably stem tissue does not negate the activity of gramicidin S to the same degree and therefore *B. brevis* WT treatments (which contain gramicidin S) are more effective.

Table 1. *Botrytis cinerea* infection of leaves and stems of tomato and disease control with *Brevibacillus brevis* – Scotland, 1998 (leaf) and 1999 (stem).

	Leaf		Stem	
Treatment	Disease severity[1] (%) ± SEM[2]	Disease control (%)	Plants with lesions (%) ± SEM[2]	Reduction in plants with lesions (%)
Control	30.6 ± 5.7 b	–	83.3 ± 12.9 a	–
B. brevis WT	17.2 ± 2.6 a	44	30.0 ± 12.2 b	64
B. brevis E-1	17.4 ± 1.3 a	43	59.3 ± 14.3 c	29
Rovral	19.9 ± 2.0 a	35	–	–

[1] Disease severity assessed by visual assessment of the percentage of leaf tissue with grey mould symptoms. [2] Values in the same column with the same letter are not significantly different (LSD at P=0.05).

Table 2. *Botrytis cinerea* infection of winter lettuce and disease control with *Brevibacillus brevis* – Scotland, 1997.

Treatment	Disease severity (%) ± SEM [1]	Disease control (%)
Control	17.8 ± 3.2 b	–
B. brevis WT	5.3 ± 1.4 a	70.2
B. brevis E-1	6.1 ± 3.4 a	65.7
Rovral	1.1 ± 0.1 c	93.8

[1] Values with the same letters are not significantly different (LSD at P=0.05)

Lettuce crop

Lettuce is a leaf crop which is subject to high yield losses from *B. cinerea*. In the UK it is grown in greenhouses and polytunnels to extend the growing season as a winter or spring crop. A winter lettuce trial with *B. brevis* WT and E-1 (4 sprays at 14 d intervals from 6 d after planting) gave good protection against grey mould with disease control levels of 65-70% (Table 2). There was no significant difference between *B. brevis* WT and E-1 indicating that the biosurfactant mode of action was operative. In the following spring lettuce trial, a pre- and post-harvest disease score was made since apparent disease pre-harvest, although varying considerably, was only about 12% with reduced or no protective activity by the *B. brevis* treatments (8% and 12% severity with WT and E-1 respectively) compared to the winter trial.

Post-harvest disease assessment revealed that in the humid environment of the lower leaves, where *Botrytis* disease commonly initiates to give basal rot, disease was considerable (25% severity). Treatment with *B. brevis* WT was most successful in reducing this disease (as low as 8% disease severity) whereas E-1 treatments were much less effective with disease severity levels as high as 23%. The data suggests that in environments where periodic wetting and drying takes place (i.e. upper surface of lettuce leaves), protection, when afforded, is via activity of the biosurfactant. In contrast, when high humidity predominates and surfaces remain wet, the biosurfactant cannot operate and it is gramicidin S that gives protection.

Cucumber powdery mildew

Powdery mildews do not generally require surface moisture for infection and so biocontrol by biosurfactant activity is not expected. Conidia of cucumber powdery mildew, *Sphaerotheca fuliginea*, were tested with the antifungal metabolite gramicidin S. Germination of conidia (*in vitro* on glass slides) was inhibited (80%) by concentrations as low as 1-5 µM. When *B. brevis* WT cultures were tested for efficacy in controlling *S. fuliginea* infection of young cucumber plants under greenhouse conditions (23°C, 70% RH, 18h light, 6h dark), 40% disease control was achieved (Seddon & Schmitt, 1999) (see also Integrated disease control).

Damping-off

Damping-off diseases are caused by a range of fungal species including *Pythium* and, to some extent, *B. cinerea*. *In vitro* studies have shown that *B. brevis* WT but not E-1 inhibited pathogenic *Pythium* species associated with damping-off disease (*P. aphanidermatum*, *P. mamillatum* and *P. ultimum*) (Walker *et al.*, 1998). When tested on seeds of peas and dwarf French beans germinating on water agar, *B. brevis* WT was shown to protect against damping-off disease caused by *P. mamillatum* and *B. cinerea* in most cases. The level of disease control (80-100%) was greatest against *B. cinerea* but more variable (30-65%) against *P. mamillatum*. Two lots of dwarf French beans were tested, white coated and black coated seeds and no protection against damping-off was observed by *B. brevis* treatment with black coated seeds. The number of viable propagules of *B. brevis* in this spermosphere declined considerably in comparison to the other seed lots and may be the reason for the loss in disease control (Walker, 1995). This is an interesting and important observation for seed treatment strategies and biocontrol purposes.

Post-harvest diseases of fruits

Considering the range of plant pathogens inhibited by *B. brevis*, there is potential for its use as a BCA with post-harvest diseases. The environment in post-harvest situations (stores, transport and outlet points) can be controlled by the operator (i.e. RH and temperature) and therefore it may be possible to match cultural practices to support the BCA and suppress the pathogen. Preliminary studies have been made with fruits (pears and grapes). With wounded pears infected with *B. cinerea* (10^3-10^4 conidia ml^{-1}), dip-treatments with *B. brevis* (10^8 cell ml^{-1}) reduced lesion diameters when scored after further incubation for 7 days at 25°C. Both *B. brevis* WT and E-1 were shown to be effective in reducing lesion diameter (35-55% and 30-75% efficacy respectively) (Adie, 1996). Gramicidin S treatment (100 µM) also suppressed wound infection (about 60% efficacy). Since *B. brevis* WT, E-1 and gramicidin S all showed suppression further studies were made with the *B. brevis* WT strain only on table grapes (Ellis, 1996). Grey mould infection gave levels of 20% and 90% grapes diseased for red and green varieties of table grapes respectively. Control of disease with *B. brevis* was only achieved with green grapes (90% disease control). It is interesting that in two plant food product systems (grapes and beans) the presence of coloured material, possibly tannins, polyphenolic compounds and other pigments, appears to interfere with microbial activity in a non-specific manner (both pathogen and BCA affected) and an awareness of such subtlety is necessary when developing these BCAs.

Integrated disease control

As indicated, biocontrol with *B. brevis* can be variable depending on the crop system and environmental parameters. When efficacy values are substantially lower than effective fungicides it may be possible to integrate two or more biocontrol systems and achieve high levels of disease control. In young cucumber plants, when *B. brevis* was combined with plant extracts of *Reynoutria sachalinensis*, that have been shown to induce resistance in cucumber (Daayf *et al.*, 1995), levels of disease control of about 90% were achieved against *S. fuliginea*. *B. brevis* treatment alone gave about 35% disease suppression and plant extracts of *R. sachalinensis* 65% (Figure 1). The combined treatment therefore was additive and as effective as standard fungicides in controlling cucumber powdery mildew.

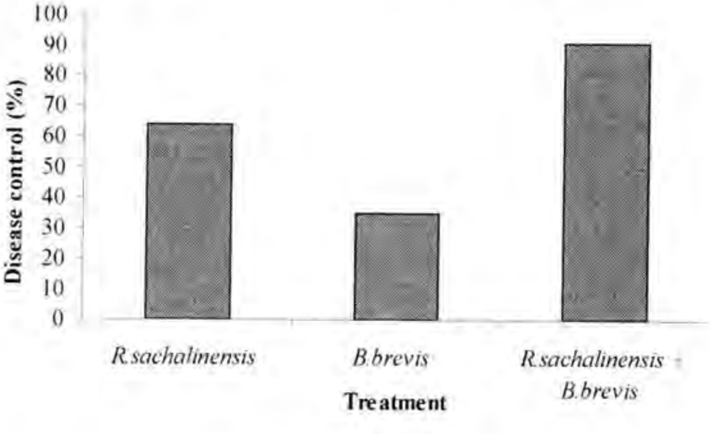

Figure 1. Effects of *B. brevis* and *R. sachalinensis* plant extract treatments alone and in combination on disease control of *Sphaerotheca fuliginea*. *B. brevis* treatment one day after inoculation with *S. fuliginea* and *R. sachalinensis* four days before.

An alternative strategy is to combine *B. brevis* with the use of fungicides. Many fungicides however strongly inhibit the BCA (e.g. dichlofluanid and chlorothalonil) so care is needed in making this combination whether in mixtures or in alternating programme strategies. Iprodione and benomyl inhibited *B. brevis* at levels higher than the recommended dose but not at recommended rates when tested *in vitro* so this combination could prove useful for integrated disease control (Seddon *et al.*, 1996).

DISCUSSION AND FUTURE WORK

B. brevis has potential as a BCA in a range of cropping systems and against several fungal plant pathogens. With more than one mode of antagonism, resistance to this BCA is unlikely to develop in the crop environment. Although *B. cinerea* has been the main target of this BCA, its use against powdery mildews, damping-off, post-harvest and other diseases warrants further investigation. Depending on the situation, the level of control may be sufficient to control the pathogen (e.g. 90% in table grapes) but in others it may not (e.g. 40% in tomato crops) and integration of this BCA with other biological methods and/or with cultural practices, or fungicides, may be necessary (Seddon *et al.*, 1996; Seddon *et al.*, 1997; Seddon & Schmitt, 1999).

Ecotoxicology tests concerning safety to the environment (and also humans) are needed prior to the use in practice of *B. brevis* and these studies are underway. Two areas where further research should prove fruitful are (i) formulation requirements and nutrient supplementation of the BCA inoculum and (ii) application rates and times. Situations where 65-70% disease control have been observed (e.g. lettuce crop) may well give rise to successful disease control levels of 90% and higher when these parameters have been determined to favour biocontrol. Increasing the activity of the BCA might be achieved by altering the growth media and/or growth conditions. Little work has been done on the survival and longevity of *B. brevis* in the crop environment. Additives (germinants) could be used to activate the dormant spore once it is introduced (Seddon *et al.*, 1997). Many plant surfaces (stems, leaves) have only limited nutrient availability and introducing the BCA in a high dose at one time may not be the best way for its establishment. More frequent applications of lower dosage could well sustain *B. brevis* activity and lead to higher disease control. For biological pest control in greenhouse crops, routine introduction of parasites and predators is practiced throughout the season. Altering the environmental parameters, if this is possible, to bias conditions against the pathogen and in favour of the BCA could also lead to efficient and effective disease control. The most favourable conditions for *B. brevis* are those that allow periods of interrupted wetness and can be achieved with environment-controlled greenhouses but would be difficult to control in open field situations or unheated polytunnels. No data is available as yet but in future *B. brevis* should be tested with field crops and with other important fungal plant pathogens such as *Phytophthora infestans* in potato crops and *Blumeria graminis* in cereal crops.

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SESSION 5B

ADVANCES IN APHID MANAGEMENT

Chairman	Professor R Blackshaw *University of Plymouth, UK*
Session Organiser	Dr K F A Walters *Central Science Laboratory, York, UK*
Papers:	5B-1 to 5B-5

The field scale distribution of insects in winter wheat

L Winder, C Woolley
Seale-Hayne Faculty, University of Plymouth, Newton Abbot, Devon, TQ12 6NQ, UK

J M Holland
The Game Conservancy Trust, Fordingbridge, Hampshire, SP6 1EF, UK

J N Perry, C J Alexander
IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK

ABSTRACT

The work described in this paper characterises the spatial and temporal distributions of aphids and their natural enemies in winter wheat. Three uses of these data are presented, which investigate: firstly, the possible effectiveness of precision-based spatially varied pesticide application; secondly, crop-scouting strategies; and thirdly, predator-prey interactions. Results from field studies describing the development of populations of *Sitobion avenae* and *Metopolophium dirhodum* are presented and the implications for the development of Integrated Pest Management strategies discussed.

INTRODUCTION

The development of reliable aphid-control strategies is an important component of Integrated Pest Management. Information regarding the field-scale distribution of aphid populations, both in time and space, could improve our ability to apply pesticides optimally and also increase our understanding of natural pest control. Until recently, there have been few spatially-explicit studies that describe or investigate field-scale insect distributions. Recent studies have demonstrated pattern both spatially and temporally, both for aphids and their natural enemies (Winder *et al.*, 1999; Holland *et al.*, 1999) whilst Murchie *et al.* (1999) showed that the quantification of insect spatial distribution might be used to develop precision targeting of insecticides. These studies used the new methodology of Spatial Analysis by Distance Indices (SADIE), developed to describe two-dimensional spatially-referenced count data (Perry *et al.*, 1996, 1999).

In this paper we present three examples of how information regarding the spatial and temporal distribution of insects can be of value for the development of Integrated Pest Management. The first example investigates the spatial distribution of aphids; the conclusions are relevant to precision insecticide applications. The second example illustrates how such spatial information may be used to improve crop-scouting and optimise spray applications. The third example demonstrates how spatial data may be used to investigate the relationships between prey populations and their natural enemies. We discuss these findings in the context of Integrated Pest Management.

METHODS AND MATERIALS

Intensive sampling of a 5.7 ha field of winter wheat (Coffinswell, Newton Abbot, Devon, UK) was conducted on five occasions during 1999: 27 May, 7, 21 June, 5 and 19 July. A two-dimensional 16 x 16 grid comprising 256 sampling locations was positioned within the crop with a spacing of 12m. At each location aphid numbers and species were recorded by inspecting 25 tillers. Barrier-connected pitfall traps (Winder *et al.*, in press) were used to record ground-active beneficial insects.

Data were analysed by calculating the SADIE index of aggregation, I_a, and its associated probability level, P_a; values of I_a around unity indicate a random arrangement of the observed counts, values larger than unity indicate spatial aggregation. SADIE cluster indices for each sample unit were contoured into red-blue plots; areas where the estimated degree of clustering was half as great again as that expected by chance were defined as clusters. Clusters are either patches where neighbouring units all had greater than average counts, or gaps where neighbouring counts were all smaller than average.

Additionally, regressions were done of aphid rate of increase (r_t) against predator activity-density, recorded as the numbers of beetles caught in pitfall traps. Aphid rate of increase was calculated from:

$$r_t = \frac{\ln(n_t + 1) - \ln(n_0 + 1)}{t}$$

where, n_t and n_0 represent the aphid counts at the end and the beginning of time interval t, respectively.

RESULTS

Two aphid species, the grain aphid *Sitobion avenae* and the rose-grain aphid *Metopolophium dirhodum* predominated; for both species peak population was recorded on 21 June (Figure 1).

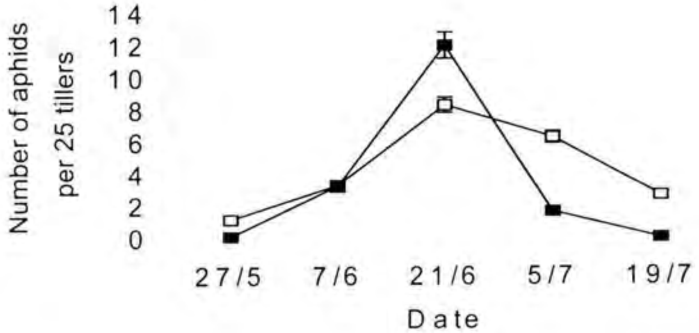

Figure 1. Arithmetic mean aphid counts (± 1 standard error) for entire field on each of the five sample dates. Counts from 25 tillers recorded at 256 locations. Filled and open squares represent *Metopolophium dirhodum* and *Sitobion avenae*, respectively.

Analysis of spatial pattern showed considerable spatial aggregation on most dates (Table 1). For *S. avenae*, gap clusters were considerably larger than patch clusters in the red-blue plots (Figure 2). Individual patches covered areas up to approximately 1000m^2, although most were considerably smaller. Similar patterns were evident for *M. dirhodum*. For both species, the location of patches was ephemeral.

Table 1. Degree of spatial pattern for two aphid species and two ground-active predatory beetles, over five sample dates.

	Sitobion avenae		*Metopolphium dirhodum*		*Pterostichus melanarius*		*Pterostichus madidus*	
	I_a	P_a	I_a	P_a	I_a	P_a	I_a	P_a
27 May	1.28	0.0548	1.15	0.1668	1.50	0.0070	0.99	0.4615
7 June	2.21	0.0002	1.33	0.0334	4.27	0.0002	1.19	0.1171
21 June	1.17	0.1222	1.82	0.0002	1.78	0.0002	2.12	0.0002
5 July	1.24	0.0736	0.88	0.7836	1.84	0.0002	1.77	0.0002
19 July	1.11	0.1967	1.29	0.0639	2.66	0.0002	1.71	0.0003

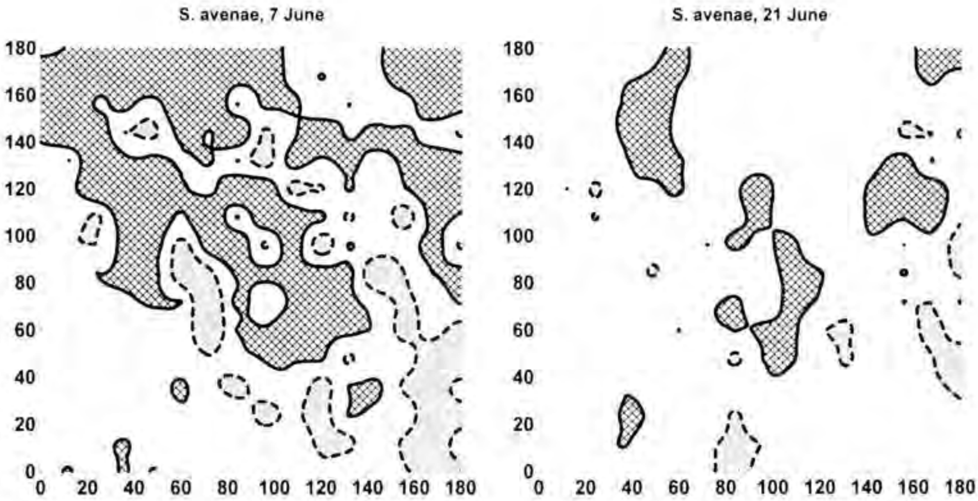

Figure 2. Red-blue plots for *S. avenae* on 7 and 21 June. Clustering indices for each of 256 units contoured into areas of strong clustering, patches bounded by dashed contours, gaps in hatched areas bounded by solid contours.

Simulation was used to study four possible crop-scouting strategies, using as an example the observed data for *M. dirhodum* on 21 June. Each of the strategies: a square, a line transect, a cross and a random sample, was compared for sample sizes (N) of 9 and 16 units. For the square, line transect and cross, the sampled units were contiguous to each other. After the position of the centre of each such arrangement was found for each simulation by random placement, the other units to be sampled were determined unambiguously. By contrast, the random sample, by definition, required each sample unit to be chosen randomly and independently of all others in the sample. The geometric mean count per sample was calculated from each of 32 simulations of each strategy. This was compared to the true value of 6.58, derived from all 256 observed counts. All strategies, as expected, provided reasonable estimates of the true geometric mean when assessed over all 32 simulations. However, a more stringent criterion to measure the efficiency of each strategy was the percentage of simulations for which its estimate was within 5% of the true value. Not surprisingly, better estimates were derived for samples of N = 16 units than from N = 9 (Table 2). When N = 9 units were sampled, the random sample and cross strategy proved to be the most reliable, whilst for N = 16 units, the random, cross and line transect methods were comparable. Hence, with constant sampling effort the reliability of a population estimate was dependent upon the way in which the crop was traversed.

Theoretically, a random sample should always be the most accurate. The reason for the reduction in accuracy in non-random samples, in which units are contiguous, is that such arrangements must often coincide with a patch (or gap), yielding a misleadingly large (or small) estimate. For example, the very poor performance of the 4x4 square of N = 16 units occurred because it was frequently located entirely within a patch or gap (see Figure 2). Since all line transects of N = 16 units would straddle several patches and gaps there would be less reason for a biased estimate to result, as confirmed by Table 2.

Table 2. Comparison of four crop-scouting strategies on efficiency of simulated estimates of geometric mean, for *M. dirhodum* density on 21 June at two sample sizes. The true geometric mean over the entire field (N = 256) was 6.58. The value shown is the average of the estimates over 32 simulations; the number in brackets is the percentage of the 32 simulations for which the sample estimate was within 5% of the true value.

Strategy	N = 9	N = 16
Square	5.77 (6.3)	6.60 (3.1)
Line transect	7.16 (3.1)	6.91 (18.8)
Cross	5.94 (9.4)	6.27 (18.8)
Random sample	7.39 (12.5)	6.75 (15.6)

A wide range of predatory ground and rove beetles, and lycosid and linyphiid spiders were caught during the study. Data from the predatory ground beetle genus *Pterostichus* are included here as an illustration. Both *P. melanarius* and *P. madidus* were considerably aggregated (Table 1). The effect of *P. melanarius* on the population dynamics of *S. avenae* and *M. dirhodum* was confirmed by significant regressions of intrinsic rate of aphid increase (r_m) on predator activity-density (Table 3). This was particularly evident for *M. dirhodum* between 21 June and 5 July, where there were negative relationships between the variables, indicating that the predatory beetles had a measurable retardant effect on the rate of aphid population increase, and providing evidence of their value as biological control agents.

Table 3. Regressions of intrinsic rates of aphid increase between consecutive sample dates on *Pterostichus melanarius* pitfall trap catches. Subscripts indicate sample occasion. $ME = \log_{10}$(*Pterostichus melanarius*).

r_m	*Sitobion avenae*		*Metopolophium dirhodum*	
27/5 to 7/6	$r_m = 0.055 - 0.049\ ME_{27/5}$	P=0.069	$r_m = 0.129 - 0.010\ ME_{27/5}$	P=0.675
	$r_m = 0.100 - 0.044\ ME_{7/6}$	P=0.039	$r_m = 0.168 - 0.038\ ME_{7/6}$	P=0.038
7/6 to 21/6	$r_m = 0.042 + 0.018\ ME_{7/6}$	P=0.281	$r_m = 0.086 + 0.001\ ME_{7/6}$	P=0.957
	$r_m = 0.063 + 0.003\ ME_{21/6}$	P=0.843	$r_m = 0.082 + 0.010\ ME_{21/6}$	P=0.613
21/6 to 5/7	$r_m = -0.012 + 0.008\ ME_{21/6}$	P=0.592	$r_m = -0.098 - 0.048\ ME_{21/6}$	P=0.016
	$r_m = -0.018 + 0.010\ ME_{5/7}$	P=0.374	$r_m = -0.093 - 0.031\ ME_{5/7}$	P=0.041
5/7 to 19/7	$r_m = -0.048 - 0.007\ ME_{5/7}$	P=0.492	$r_m = -0.105 + 0.012\ ME_{5/7}$	P=0.419
	$r_m = -0.042 - 0.019\ ME_{19/7}$	P=0.114	$r_m = -0.081 - 0.020\ ME_{19/7}$	P=0.188

DISCUSSION

The analyses presented in this paper illustrate the use of spatially-referenced data. In this study, there is both spatial and temporal pattern evident that could influence the development and efficiency of Integrated Pest Management systems. Aphids were spatially aggregated within ephemeral patches; these patches shifted around the field markedly throughout the sampling period.

If the population dynamics of discrete within-field aphid populations was mediated primarily by initial aphid infestations, then spatial pattern would be largely dependent on the location of initial aphid foci, due to aphid immigration or over-wintering. Populations would then develop in those locations, statically throughout the season. However, this was not the case in this study. The processes that mediate the development of actual temporal and spatial patterns are poorly understood.

Local-scale (i.e. <1m) changes in pattern may be due to the mobility of aphids, perhaps due to disturbance caused by strong gusts of wind, large rain droplets (Mann *et al.*, 1995) or natural enemies. Field-scale changes in pattern may be due to a number of factors, possibly including natural enemies. In this study we show that aphid population increase is related to predator numbers, which are themselves patchy in distribution. The effect of many natural enemies, including predatory beetles, spiders and parasitoids, may result in a spatially dynamic system. Whatever the cause, these results describe a system where the location of aphid patches was unpredictable.

Our studies show that the spatial arrangement of counts influences the reliability of population estimates. However, it must be remembered that although the random sample is theoretically the most accurate, the expected time taken to traverse the crop for this strategy exceeds the others, for which sampling takes place over a strictly limited extent. In the worst case, the distance traversed for the random sample would equal the perimeter of the field. There is a trade off between the reduction in efficiency for the competing strategies and their speed of execution. When spatial pattern is present, then strategies that ignore this information are never optimally efficient. In particular, strategies based solely on mean/variance statistics or incidence, without regard for location, cannot provide the most effective approach. Currently, crop scouting is often undertaken by basing assessments on counts during a 'W' or similar shaped traverse through the crop. This study suggests that such a strategy may be a sensible compromise between a random sample and a block sample; further investigation of sample size may be warranted for cereal crops and their aphid pests.

The generalist predator *P. melanarius* was shown to influence the intrinsic rate of increase of both *S. avenae* and *M. dirhodum* populations. It is likely that other species within this group may also influence aphid population dynamics. Other spatial studies imply that such predators may have an important effect on the spatial distribution and population dynamics of their prey (Bohan *et al.*, 2000). The examples given in this paper demonstrate the importance of investigating such systems using spatially-referenced data. More understanding of processes leading to spatial pattern could increase our understanding of ecosystem processes and allow the development of more reliable decision-making strategies, as part of Integrated Pest Management systems.

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The use of field margins in the manipulation of parasitoids for aphid control in arable crops

W Powell
IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK

ABSTRACT

Parasitoids are one of the key natural enemies of aphids on arable crops. Ecological studies in cereal crops have highlighted the importance of early season synchrony between colonising aphids and their parasitoids for preventing the development of economically damaging aphid populations in the summer. To promote this synchrony, a strategy has been devised based on the manipulation of natural parasitoid populations using aphid sex pheromones. Laboratory and small-scale field studies of parasitoid foraging behaviour have shown that female aphid parasitoids from a range of economically important species respond strongly to plant-derived aphid sex pheromones and that the presence of pheromones have the potential to greatly increase levels of parasitism in aphid populations. It is proposed to use the pheromone to establish overwintering parasitoid reservoirs in field margin strips, which will be designed to provide suitable hosts and winter shelter. Some of the field margin options being promoted in current agri-environment schemes to encourage biodiversity could provide suitable habitats for the parasitoids and for other beneficial insects, giving an added, positive benefit for the farmer. This parasitoid manipulation strategy is being tested on commercial fields as part of a SAPPIO Link research project beginning this year.

INTRODUCTION

Biological control is a central component of Integrated Pest Management (IPM), and in arable crops this principally involves the exploitation of natural populations of parasitoids, predators and entomopathogens. This approach has been termed 'conservation' biological control to distinguish it from 'classical' biological control, in which species are introduced into regions where they did not previously occur, usually to combat an introduced pest, and 'augmentation' biological control , in which biological control agents are mass reared and released to augment natural populations.

In some U.K. arable crops (e.g. cereals), aphid pests do not always reach levels that justify chemical control, mainly due to the action of a range of naturally occurring biological control agents, including polyphagous and specialist predators, entomopathogenic fungi and parasitoids (Wratten & Powell, 1991). However, this 'natural' biological control often functions too late to prevent some economic damage to the crop, necessitating the use of insecticides. Therefore, research has been aimed at enhancing the efficiency of these biological control agents, by conserving and increasing their populations on farmland and manipulating them to ensure their presence in fields at a time when they will have maximum impact. This requires an understanding of their ecology and behaviour.

Behavioural research has concentrated on the foraging behaviour of aphid parasitoids in an effort to devise strategies for their manipulation within the arable farming ecosystem, especially by exploiting their responses to the semiochemical cues that they use to locate their hosts and host plants. Simply understanding the fundamental behavioural mechanisms and identifying the cues involved is not enough however, because to use that knowledge effectively in pest control strategies, it is essential to link it with ecological knowledge of the target system. Essentially, the 'weaknesses' in the ecology of the parasitoid, that sometimes lead to its failure as a biological control agent, need to be identified and behavioural manipulation strategies targeted specifically to overcome these problems. The development of such a strategy to manipulate parasitoids for aphid control in arable crops and the initiation of field studies to test and, if necessary, refine the strategy are outlined in this paper.

THE ROLE OF PARASITOIDS IN APHID CONTROL

Initial ecological studies were focussed on the role of aphid parasitoids as biological control agents in the cereal ecosystem, because of the importance of cereals in northern European arable agriculture and because there was considerable existing knowledge of cereal aphid ecology. It was apparent that natural biological control often played an important part in preventing summer aphid populations from reaching damaging levels, but the specific role of parasitoids and why this natural control sometimes failed, resulting in aphid outbreaks and inducing many farmers to adopt routine prophylactic spray programmes in the 1970's and 1980's, was less well understood.

At this time the use of spring-sown cereals was rapidly declining and sowing dates for winter crops were becoming earlier. This meant that many crops were being colonised by aphids in the autumn, with attendant increases in barley yellow dwarf virus (BYDV) problems. Furthermore, if the winter was mild, aphids survived on the crop until the following spring when their numbers were boosted by the spring immigration of winged aphids from other overwintering sites. However, contrary to expectations, mild winters frequently did not result in aphid problems and aphid outbreaks often followed colder winters (Powell, 1983; Wratten & Powell, 1991). Investigation of aphid populations that had survived on winter wheat through two mild winters in the early 1980's revealed that a significant proportion of them were infested by parasitoids (Table 1).

Table 1. Effect of winter temperatures on overwintering parasitoid reservoirs within winter wheat fields.

	1979	1980	1981
Day-degrees below 0°C (Nov.-March)	112.3	32.3	30.6
% Aphids in crop parasitised (April)	No Aphids	36%	30%
Adult parasitoids / sq. m. (early June)	1.7	10.5	20.2

This meant that parasitoids emerged directly within the crop in spring and so were on hand to attack the spring aphid immigrants as soon as they entered the crop. This significantly reduced the initial aphid population growth rate in spring and early summer, allowing other natural enemies in the system to maintain aphid numbers below damage thresholds throughout the remainder of the season. Therefore, the early-season synchronisation between parasitoids and colonising aphids appears to be a key factor in the natural biological control of cereal aphids and the principal is almost certainly relevant to some other field-grown crops. However, in cereals, this synchronisation is disrupted if prolonged cold periods in winter kill aphids on the exposed crops or if BYDV problems necessitate insecticide applications in autumn, thereby removing the parasitoid reservoir from the field.

Thus, the perceived weakness in the ecology of the parasitoids that contributes to the breakdown of natural biological control in this system that occurs in some years, is the lack of synchronisation with colonising aphids in spring/early summer. Can this weakness be overcome by the behavioural manipulation of parasitoid populations?

BEHAVIOURAL MANIPULATION OF APHID PARASITOIDS

As the aim of developing behavioural manipulation techniques was to ensure that the aphid parasitoids were present in the right place (i.e. in the crop) at the right time (i.e. at the time of the spring aphid immigration), it was essential to understand the foraging behaviour of the parasitoids and, in particular, to identify the sensory cues involved in host location. Parasitoids principally use chemical information to locate hosts and host plants, and are known to exploit a range of semiochemical cues originating from both the host itself, the food plant of the host and interactions between the two (Vet & Dicke, 1992; Powell *et al.*, 1998).

Some parasitoids, including those that attack sucking insect pests, such as scale insects and aphids, utilise the communication pheromones of their hosts as foraging cues (Powell, 1999). Many aphid species develop a sexual generation in autumn, in order to produce overwintering eggs, and these sexual females release a sex pheromone to attract the winged males for mating. Aphid sex pheromones have now been successfully isolated and chemically identified and a synthetic version produced, based on extracts from catmint plants (*Nepeta cataria*) (Pickett *et al.*, 1992). These plant-extracted pheromones proved to be attractive to female aphid parasitoids, the first evidence being the capture of large numbers of female *Praon* spp. in pheromone traps consisting of water-filled Petri dishes to which pheromone lures were attached (Hardie *et al.*, 1991). Subsequently, similar traps placed in early-sown winter wheat fields in autumn caught large numbers of female *Praon volucre*, a generalist species regularly recorded attacking cereal aphids in summer (Powell *et al.*, 1993).

Detailed laboratory studies of parasitoid behavioural responses to these aphid sex pheromones, using wind tunnels, olfactometers and electrophysiology, revealed that a range of aphid parasitoid species, including those with more specialised host ranges, showed positive responses (Table 2), but the response seemed to be restricted to females (Glinwood, 1998). Furthermore, parasitoids responded to the pheromones throughout the year, even though natural aphid sex pheromones would only normally be present in the field for a short period in the autumn. Experimental evidence strongly suggests that the parasitoid response is genetically determined rather than conditioned or learned through experience (Poppy *et al.*, 1997).

Table 2. Aphid parasitoid species showing flight responses to aphid sex pheromone components in wind tunnel bioassays. All species tested showed a response.

Parasitoid Species	Main Pests Attacked	Strongest Response to:
Praon volucre	Cereal Aphids	Nepetalactone
Praon myzophagum	Myzus persicae	Nepetalactone + Nepetalactol
Aphidius rhopalosiphi	Cereal Aphids	Nepetalactone
Aphidius ervi	Legume Aphids	Nepetalactone + Nepetalactol
Aphidius eadyi	Acyrthosiphon pisum	Nepetalactone + Nepetalactol
Diaeretiella rapae	Brassica Aphids	Nepetalactone
Ephedrus plagiator	Fruit Tree / Hop Aphids	Nepetalactol

To test whether these plant-derived aphid sex pheromones have potential for enhancing parasitoid efficiency in the field, a series of small-scale field trials were done using aphid-infested, potted trap plants, with and without pheromone-releasing vials attached. The presence of pheromone increased parasitisation of cereal aphids on these trap plants by 3-11 times when the plants were placed in the margins of recently-harvested cereal fields (Glinwood *et al.*, 1998). Both the generalist *P. volucre* and the cereal aphid specialist *Aphidius rhopalosiphi* were positively affected by the pheromones.

Thus, laboratory studies and small-scale field trials have shown that female aphid parasitoids from a range of economically important species respond strongly to plant-derived aphid sex pheromones and that the presence of pheromones have the potential to greatly increase levels of parasitism in the immediate vicinity of a pheromone release point (at least within a distance of 1 m (Glinwood *et al.*, 1998)). Could the pheromones be used to promote the desired early-season synchronisation between parasitoids and colonising aphids within the crop?

THE POTENTIAL ROLE OF FIELD MARGINS

Annual arable crops are temporary habitats subject to much disturbance. As crops such as cereals ripen they become unsuitable food plants for aphids, which disappear from the crop. The plant itself disappears at harvest and may be replaced by a different crop later in the year or the following spring. This forces aphid parasitoids, which have a generation time of 2-3 weeks during the summer, to disperse from the crop field in search of hosts in more stable habitats such as field margins, woodland, pasture, hedgerows and other semi-natural habitats. As a consequence, many of them pass the winter in these habitats, either in the mummy stage or in slowly developing, anholocyclic, aphid hosts, and utilise hosts in these areas in the following spring before spreading back to crop fields. This creates a time delay between aphids colonising the crop and the influx of parasitoids, which arrive in response to already expanding host populations.

To alleviate this problem a strategy for parasitoid manipulation has been devised, based on the parasitoids' known response to the plant-derived aphid sex pheromones. It is proposed to use the pheromones to concentrate parasitoids in appropriate field margin strips at the time they are dispersing in late summer/autumn, thereby establishing an overwintering reservoir adjacent to the crop (Powell *et al.*, 1998). If necessary, the pheromone could be deployed again in the spring to lure them back into the crop when colonising aphids begin to arrive. The field margin strips will need to be managed to provide suitable shelter and a diversity of aphid hosts appropriate for the parasitoid species relevant to the crops being grown in the area. In the case of cereals, the presence of tussocky grass would be particularly suitable and compatible with the requirements of other important natural enemies such as carabid beetles. A number of the field margin management options available in current agri-environment schemes, such as the countryside and arable stewardship schemes, are potentially suitable for this strategy, giving the farmer the added benefit of boosting natural aphid control while at the same time receiving support for enhancing farmland biodiversity through these schemes.

Whilst the results of the laboratory and small-scale field experiments using the sex pheromone gave very positive results, demonstrating its potential for use in parasitoid manipulation, the viability of the current proposed strategy needs to be tested at a realistic field scale. Attempts to do this have begun this year as part of a broader project investigating the use of diverse field margins for enhancing pest control and biodiversity. The project is funded under the 'Sustainable Arable Production through Precision Input Optimisation (SAPPIO)' Link programme, sponsored by MAFF and SERAD. The research is being done by IACR-Rothamsted & Long Ashton, the Game Conservancy Trust, Central Science Laboratory and the Scottish Agricultural College. Other participants and sponsors are Dow Agrosciences, the Home-Grown Cereals Authority (HGCA), the Horticultural Development Council (HDC), the Processors and Growers Research Organisation (PGRO), Unilever, United AgriProducts (UAP), CWS Agriculture, Tesco, the Chadacre Agricultural Trust, the Dulverton Trust, the Manydown Company, the Worshipful Company of Farmers and the Yorkshire Agricultural Society.

Other work in the project involves the development of appropriate wild flower mixtures for sowing in margins to provide high quality nectar and pollen sources for adult hoverflies, which need such food for the efficient development of their eggs, and the use of molecular techniques to detect and monitor levels of predation of aphids by spiders and carabid beetles, as affected by field margin and crop management. An important component is the use of new statistical techniques to measure the effects of field margin and within-crop management techniques on the spatial and temporal distribution of both pest and beneficial insects across fields of varying sizes. It is essential to demonstrate that natural enemy manipulation strategies using field margin management is having a significant impact on pest control within the crop and to establish the distance into the crop over which this impact extends.

A second LINK project, funded under the 'Competitive Industrial Materials from Non-Food Crops (CIMNFC)' programme and also sponsored by MAFF, is developing an economically viable and environmentally acceptable commercial production system for aphid sex pheromones, based on growing catmint as an industrial crop. The collaborating partners in this project are IACR-Rothamsted, AgriSense-BCS, English Hop Products and

Richard Wood Partnerships. As part of this project the pheromone has been formulated into a polymer strip, allowing controlled release, which should be cheap and simple to use.

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Opportunities for managing aphids in outdoor lettuce crops

G M Tatchell
Entomological Sciences Department, Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK

ABSTRACT

Four species of aphid (*Myzus persicae, Macrosiphum euphorbiae, Nasonovia ribisnigri* and *Pemphigus bursarius*) infest lettuce in northern Europe. The demand by consumers for high quality lettuce that is completely free from aphid infestation provides considerable challenges to the producer. Monitoring aphid numbers with water traps and on plots of lettuce planted sequentially from April to September has identified two periods when crops are particularly at risk from infestation. Temperature-based forecasts can predict these events to enable accurate targeting of some novel control strategies currently the subject of research. Aphids can be prevented from establishing on lettuce crops either by using semiochemicals to modify aphid host-finding behaviour to reduce crop colonisation, or by using resistant varieties. Should aphids colonise crops, they can be killed by insecticides, or in the case of *P. bursarius* by the use of the fungus *Metarhizium anisopliae* incorporated in modules at planting. The selection of control options for integration differs according to risk of aphid infestation.

INTRODUCTION

In Britain lettuce (*Lactuca sativa*) is grown mainly outdoors from the months of mid-March to October, but individual crops develop in six to eight weeks. Production in Britain during the remaining months is prevented by cool winters and frosts, but production is frequently transferred to southern Europe to ensure continuity of supply to the consumer. This paper addresses the crops grown outdoors in the U.K.

British consumers demand aphid-free lettuce. The presence of any aphid-infested lettuce heads in consignments to supermarkets may result in their rejection. This presents a considerable challenge to both the growers and to entomologists as four species of aphid infest lettuce regularly. Of these, *Myzus persicae* and *Macrosiphum euphorbiae* feed on the leaves of many species of plant including lettuce, while *Nasonovia ribisnigri* feeds on the leaves and *Pemphigus bursarius* feeds on the roots of lettuce in summer (Reinink & Dieleman, 1993). In addition, consumers and government policy demand safe food to which a minimum or no insecticides have been applied.

The need to control four aphid species, some of which have different life cycles, indicates that crops planted at different times during the year should not be treated in the same way if effective control is to be achieved. This is exacerbated by the small number of insecticides registered for use on minor crops such as lettuce. In addition, resistance to insecticides now occurs in *M. persicae* (Field *et al.*, 1997), *N. ribisnigri* (Barber *et al.*, 1999) and *M. euphorbiae* (Denholm pers. com.), so making those compounds that are available less effective. There is therefore a clear need to refine our knowledge of the different aphid species that infest lettuce and use this knowledge to develop and introduce novel control strategies, as well as to make best use of the insecticides that are available.

This paper gives a brief overview of recent research done to develop the components of an integrated control strategy for aphids on lettuce. The focus of the paper is on the non-insecticidal components of the control strategy, and some components are at the experimental stage.

PREDICTING PERIODS OF RISK

Determining periods of aphid colonisation and infestation

The periods in the year when crops are most at risk from aphid infestation were identified. This was achieved by 1) placing yellow water traps at ground level to monitor winged aphids and 2) planting plots of insecticide-free lettuce sequentially on five occasions (April to September) throughout the period of crop growth, to monitor aphid colonisation and population development. Data were collected over four seasons from areas representative of most of the lettuce production in the U.K. (Collier *et al.* in prep.)

By combining data from the sequentially-planted plots and the water traps, it was possible to build a picture of the main periods of risk. The data for leaf- and root-feeding aphids indicated clearly that there were two main periods when lettuce crops were at risk from aphid infestation, in June-July and from early September onwards (Figure 1). All leaf-feeding species were present during the first period, while *N. ribisnigri* was dominant in the autumn. The root-feeding *P. bursarius* did not occur in autumn as colonising alates. It is particularly important to note that there were periods when the risk of aphid infestation was low.

Forecasting periods of risk

Accurate targeting of control strategies is possible when periods of risk can be predicted. In Britain the life cycles of the four aphid species fall into two groups. The two species that are specific to lettuce during the summer period, *N. ribisnigri* and *P. bursarius*, overwinter on different plants as eggs. In spring the eggs hatch, and after a period of development associated with temperature, winged aphids fly and colonise lettuce. The time of migration to crops can be predicted by a simple accumulation of day degrees from egg hatch (Collier *et al.*, 1994, in prep.). In contrast, the two polyphagous species overwinter as active stages on a wide range of plant species. It has been shown that the dates by which winged aphids are first detected in suction trap samples, and hence colonise crops, are related to the severity of the cold in the previous winter (Harrington *et al.*, 1990).

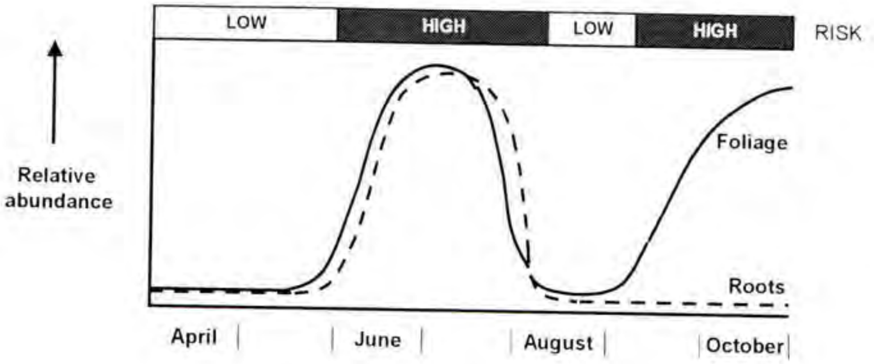

Figure 1. Periods when the risk of infestation of the foliage or roots of lettuce by aphids is high or low, based on aphid abundance in water trap samples and in samples taken from sequentially-planted plots of lettuce.

Forecasting specific life cycle events

The first type of forecast can be illustrated using *P. bursarius*. This species overwinters as an egg in crevices in the bark of poplar trees (*Populus nigra* var. *italica*). In spring the eggs hatch and the young aphids crawl onto leaves where they form a gall on the leaf petiole. Once the gall is fully formed and the aphid reaches the adult stage, it reproduces parthenogenetically. There may be as many as 200 individuals in one gall, originating from a single aphid. These aphids develop as alatae (winged aphids) which leave the gall and colonise lettuce (Dunn, 1959). On lettuce, the alatae produce nymphs by parthenogenesis. The nymphs then crawl to the roots to feed, mature and reproduce. From careful analysis of data for the first appearance of alatae in galls, the time of the migration from poplar to lettuce is predicted accurately by the accumulation of 672°D above a threshold of 4.4°C from the beginning of February (Collier *et al.*, 1999). This approach is possible because the timing of a specific stage in the life cycle of the aphid is being predicted. Such a forecast enables specific strategies to control this species to be timed accurately.

Associations between weather and abundance

The second type of forecast involves associations between specific events in the biology of aphids and weather data (e.g. the occurrence of first aphid in the trap samples). Such associations have been derived from the data set collected by the Rothamsted Insect Survey over 30 years. A network of suction traps throughout Britain has monitored daily the numbers of each aphid species dispersing to crops. For species that overwinter as anholocyclic clones, rather than as eggs, the date on which the first aphid is found in the trap sample is inversely related to the winter temperature i.e. the colder the winter the later the aphids fly to crops (Harrington *et al.*, 1990). These relationships explain well the considerable variation between years in the timing of the first occurrence of winged *M. persicae* and *M. euphorbiae* on lettuce.

APPROACHES TO APHID CONTROL

Having predicted when aphids will colonise crops, it is constructive to implement control strategies based on those predictions. Longer-term forecasts can be used to time control strategies requiring treatment at or before planting, e.g. the use of aphid-resistant varieties. Shorter-term forecasts may be used to time insecticides during crop growth. There are two approaches to aphid control. Firstly, aphids can be prevented from colonising crops, and, secondly, they can be removed if populations become established.

Prevention of aphid establishment

Plant volatiles to alter insect host-finding behaviour

A second approach to preventing aphids from establishing on plants is to modify their host-finding behaviour. Aphids use a combination of sight and olfaction to locate their host plants. Species of aphid that alternate between different host plant species in summer and winter have to be able to respond to the two plant types in different ways, depending on the season. *Nasonovia ribisnigri* spends the winter as an egg on the primary host, blackcurrant (*Ribes nigrum*). Once eggs hatch in spring, the aphids develop winged forms that migrate to secondary hosts, including lettuce. During this phase *N. ribisnigri* are attracted to lettuce but not to blackcurrant. It is hypothesised that, when winged aphids are in search of their secondary host, the presence of volatiles from the primary host will mask their attraction to the secondary host. Attempts are being made to reduce colonisation of lettuce by *N. ribisnigri* by releasing volatile chemicals from blackcurrant over lettuce plants (c.f. Petterson *et al.*, 1994).

In collaboration with Rothamsted Experimental Station, the volatile chemicals to which *N. ribisnigri* responds have been identified. The volatile chemicals were extracted from blackcurrant and lettuce and the response of alate *N. ribisnigri* to single compounds was determined by coupled gas chromatography and electrophysiological recording from single olfactory cells in the antennae (Wadhams, 1990). The compounds were identified by GC-MS.

Nasonovia ribisnigri showed a very strong electrophysiological response to lettuce. However, the compounds which elicited the response occurred in such low concentrations that identification has not yet been possible. In contrast, the eleven compounds identified from blackcurrant to which *N. ribisnigri* responded electrophysiologically have been identified. However, an electrophysiological response does not indicate a behavioural response. This needs to be quantified by further experimentation.

A Petterson olfactometer (Petterson, 1970) was used to quantify the behavioural responses of alate *N. ribisnigri* to the volatile compounds produced by lettuce and blackcurrant in a series of progressively more detailed experiments. Firstly, the response to whole leaves was determined; alate *N. ribisnigri* were attracted by the volatiles from a lettuce leaf and repelled by those from a blackcurrant leaf. Attraction to a lettuce leaf was masked in the presence of a blackcurrant leaf. Secondly, the eleven compounds from blackcurrant, identified from the electrophysiological studies, were assayed individually in the Petterson olfactometer and the behavioural responses compared with those to lettuce alone. The responses to individual compounds varied considerably, with a few acting as attractants. However, *N. ribisnigri* showed no significant response to most of them. Interestingly, no individual compound acted as a repellent. This indicated that no compound alone could be developed to modify behaviour. Therefore a synthetic mixture of the eleven physiologically-active compounds was made. The response of *N. ribisnigri* to this mixture alone and in the presence of lettuce was determined in the olfactometer. The synthetic mixture elicited a very similar repellant response to that to the "whole" volatiles from blackcurrant leaves. In addition, the synthetic mixture masked the attraction to lettuce in a similar way to the whole blackcurrant leaves.

The positive results from olfactometer studies provided encouragement to test the volatiles in field experiments. In small cage experiments, the numbers of alate *N. ribisnigri* colonising lettuce seedlings grown in trays, over which were suspended sachets releasing the synthetic mixture of blackcurrant volatiles, were reduced as compared to untreated trays in the same cage. A novel experimental design that accounted for the dispersal of volatiles by wind was used in further studies. Again lures were suspended over the treated plots of young lettuce. Large numbers of winged *N. ribisnigri* were released into the experiment from heavily infested plants raised in a glasshouse and placed around the outside of the experiment. At harvest, after six weeks, the aphids were identified and counted. Again there was a small, but statistically significant reduction in the numbers of *N. ribisnigri* on treated plots. The reductions achieved are not yet sufficient to be of commercial benefit. However, if the ratio of compounds in the synthetic mixture and the release rates can be optimised, there may be potential for commercialisation. These results do provide encouragement for the development of this novel component in an integrated crop management package for the future.

Resistant varieties

Research by HRI and in the Netherlands has identified sources of antibiosis resistance to aphids in lettuce and wild species of *Lactuca*. Most effort has been directed towards breeding for resistance to *P. bursarius* and *N. ribisnigri* as these are the two species that are specific to lettuce. Commercial varieties that are resistant to *P. bursarius* (Ellis *et al.*, in press) or *N. ribisnigri* (Aarts *et al.*, 1999) are now available, although there are, as yet, no commercial varieties resistant to both species. The deployment of these varieties has to be handled with care as the resistances are based on single dominant genes and it is possible that genotypes of aphids may develop that can overcome them. It is therefore vital to plant these varieties only at times when crops are particularly at risk and not to rely solely on resistance to achieve control. The forecasts described previously can be used to identify the crops that should be planted with resistant varieties. For example, in the U.K. varieties of iceberg lettuce resistant to *N. ribisnigri* tend to be grown during the period of high risk in early autumn (Figure 1).

Removal of aphids from plants

Insecticides

The use of synthetic insecticides still has a key role to play in the control of aphids on lettuce. However, the number of active ingredients available for use on lettuce is limited and this is a continuing concern to lettuce grower. Studies have demonstrated the potential of some relatively new active ingredients for controlling aphids on lettuce including imidacloprid as a seed treatment and triazamate as a foliar spray (Parker & Blood-Smyth, 1996). The former product now has Specific Off Label Approval for use on outdoor lettuce and has become the mainstay of aphid control on lettuce in the UK. Triazamate will not be registered for use on lettuce in the U.K.

Biological control

At HRI, an isolation of the fungus *Metarhizium anisopliae* has been identified for the control of *P. bursarius*. The fungus can be mass-produced on split wheat-based media and then incorporated into the block compost when the seeds are sown. When the plants are transplanted in the field, the fungus is already present close to the roots of the plant before it is colonised by aphids. The fungus infects colonising *P. bursarius* leading to disease epidemics and achieves good aphid control when compared with plants grown in blocks (Chandler, 1997). In this instance complete control is not required since the roots are not marketed and it is only necessary to prevent plant growth from being impaired. The commercialisation of such biocontrol agents remains a challenge due to the relatively high cost of registering such a product for a niche market (Tatchell, 1997).

INTEGRATED MANAGEMENT

The individual management and control options identified above (excluding volatile chemicals which were at a too preliminary stage of investigation) provide a menu from which to select components of a control strategy to manage the four aphid species found on lettuce crops planted on a defined date. A number of such strategies were devised and tested during periods of high or low risk (Figure 1) in different regions of Britain. These integrated strategies were tested against twice-weekly applications of pirimicarb on the susceptible lettuce variety Saladin and an untreated control (also Saladin). The success of each strategy was measured by the numbers of lettuce heads infested by aphids at harvest and the numbers of insecticide applications made. Nearly all strategies were effective, resulting in a greater proportion of aphid-free lettuces and fewer applications of insecticide than the pirimicarb-based control. Resistant varieties made a significant contribution to control when crops were challenged only by the species to which plants were resistant. Imidacloprid seed treatments and foliar applications of triazamate were particularly effective, in some instances reducing the number of infested lettuces from a total infestation to less than 20% of plants infested. The total number of applications of insecticide could be reduced considerably, from 14 on the pirimicarb-based control to four or less in some integrated treatments, particularly if crops were harvested during periods of low risk.

Greatest reductions in the numbers of sprays were achieved when applications were made as a managed response to the presence of aphids on lettuce plants

DISCUSSION

Consumers demand supplies of crops throughout the year For fresh fruit and vegetables this is achieved by the careful choice of crop variety, the manipulation of planting dates and the production of crops in different regions of the world The consequence of the first two of these options, particularly for short-season crops which take only two months to reach maturity, is that crops planted at different times in the same geographical location within a single annual cycle may be subject to very different risks of pest infestation In temperate regions of the world, the life cycles of insect pests are linked closely to the seasonal changes in climate These factors greatly complicate the development of integrated systems of pest management as each planting of a crop may require a different suite of control options to achieve sustainable pest control. The control of aphids on lettuce, as demonstrated here provides such an example

The determination of the annual cycle of risk of infestation, and the drivers for this, are central to the development of effective control strategies The selection of control options may be strategic and made prior to sowing, or tactical and taken during the growth of a crop. For example, strategic decisions to use aphid-resistant varieties or insecticidal seed treatment are made during early planning, while tactical applications of insecticides are made in response to the detection of an aphid infestation However, the choice will remain limited by the "menu" of options available In the short term there are three components that are dominant in current systems of lettuce production - effective insecticides, resistant varieties and accurate forecasts However, resistance to insecticides in the species that infest lettuce and the demand from consumers for fresh produce grown without the use of agrochemicals is driving the search for novel solutions

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Improved Strategies for Aphid Resistant Transgenic Crops

A M R Gatehouse, and R E Down
Department of Agriculture and Environmental Science, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK

J A Gatehouse
Plant Molecular Biology Group, Department of Biological Sciences, University of Durham, South Road, Durham, DH1 3LE, UK

P Christou
John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK

ABSTRACT

Despite the synthesis of improved pesticides, and integrated pest management strategies combining the use of chemicals, resistant germplasm and the modifying of agricultural practices, yield losses due to insects have actually increased slightly for most crops over the last two decades. The ability to introduce new genes into plants via recombinant DNA technology has resulted in a new approach to insect pest management in agricultural crop production, with the recent commercialisation in US of genetically enhanced corn (maize), potato and cotton plants expressing a gene encoding the entomocidal toxin from *Bacillus thuringiensis* (Bt).

An alternative, and often complementary strategy to the use of genes encoding Bt toxins, is in the use of genes encoding plant-derived insecticidal proteins; this is particularly pertinent for the control of economically important insect pests such as aphids and other homopteran species for which effective Bts have not been identified. A gene encoding the lectin from snowdrop (*Galanthus nivalis*; GNA), when expressed in rice was shown to significantly enhance levels of resistance towards brown planthopper, a major homopteran pest of this crop, with a 40% reduction in hopper survival. Although expression of this, and other plant-derived transgenes, in potato has been demonstrated to reduce the population build-up of aphids, the levels of protection conferred are not commercially viable. Other strategies for aphid control based on recombinant DNA technology will be addressed. The potential for aphids to act as "carriers" for plant defensive proteins thus affecting predators at the tritrophic level, will also be discussed.

INTRODUCTION

With a projected increase in world population to 10 billion over the next four decades, an immediate priority for agriculture is to achieve maximum production of food and other products. Unfortunately the price for achieving such levels may be high, with irreversible depletion or destruction of the natural environment making certain agricultural practices unsustainable in the longer term. Whilst pesticides are very effective in combating the immediate problem of insect attack on crops, and have been responsible for dramatic yield increases in crops subject to serious pest problems, non-specific pesticides are harmful to beneficial organisms including predators and parasitoids of the target pest species. In response, the agrochemical industry has introduced a number of less harmful and persistent pesticides.

The global pesticide market is in excess of $30 billion per year, with 29% of this budget being spent on insecticides. Despite this high expenditure, 15% of all crops grown are lost directly to insect damage. This fact, together with public demands for more environmentally sound methods of crop protection, and the requirements for more sustainable agricultural systems, has resulted in the development of alternative crop control strategies which are seen as forming an important component of Integrated Pest Management (IPM). One such strategy is to increase host plant resistance based on the use of genetically modified crops expressing insect resistance genes. The emergence of technologies that have allowed plants to be stably transformed with foreign genes has been timely, with the commercial introduction in US of crops expressing a gene encoding the bacterial endotoxin from *Bacillus thuringiensis* in 1996. Since their introduction several different Bt expressing crops have become commercially available, including cotton (expressing cryIA(c) for resistance to bollworm), maize (expressing cryIA(b) for control of corn borer) and potato (expressing cry IIIA for control of Colorado potato beetle). In 1999, 22% of all transgenic crops grown were modified for enhanced levels of insect resistance with a further 7% engineered for both herbicide and insect resistance, representing 9.1 and 2.9 million hectares respectively (ISAAA Briefs, 2000). However, it is note worthy that as yet there are no convincing examples of Bt expressing crops with enhanced levels of resistance to homopteran pests.

ENGINEERING CROPS FOR RESISTANCE TO HOMOPTERA

In an attempt to increase the spectrum and durability of resistance, other strategies based on the use of plant derived genes, such as enzyme inhibitors and lectins, are actively being pursued. One of the major targets for this research are Homoptera, including aphids. Insects within this order are responsible for significant crop losses, and whilst they are not usually responsible for serious plant damage by direct feeding, unless populations build up to high levels, they act as vectors for the transmission of numerous plant viral diseases. During the last decade significant progress has been made in the identification of proteins with insecticidal activity against homopteran pests following the initial demonstration that the lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) was toxic to rice brown planthopper (*Nilaparvata lugens*; BPH) when administered in artificial diet (Powell *et al.*, 1993; 1995). Subsequent to this work, other plant lectins were shown to be insecticidal towards a range of different homopteran pests including potato leaf hopper (Habibi et al., 1993) and aphids (Rahbe and Febvay, 1993; Rahbe et al., 1995; Sauvion et al 1996). These findings have been extended to other proteins, including inhibitors of both serine and cysteine proteases (Rahbé pres. comm.; Cowgill *et al.*, 1999). Despite this research effort, there are still relatively few examples of transgenic plants exhibiting enhanced levels of resistance to such pests (see Table 1). The first demonstration came in 1995 when Hilder *et al.* (1995) where able to show that tobacco plants expressing GNA were partially resistant to peach-potato aphid *Myzus persicae*. Subsequently other workers have demonstrated enhanced resistance to *M. persicae* and the glasshouse potato aphid (*Aulacorthum solani*) in potato plants expressing GNA. Recently Gatehouse *et al.* (1999) demonstrated enhanced levels of resistance to *M .persicae* in potato plants expressing the lectin from jack bean (*Canavalia* *ensiformis*; ConA). Other studies have shown that expression of GNA in rice caused about 40% and 53% reduction in survival of brown planthopper and green leafhopper (*Nephotettix virescens*; GLH) respectively (Rao *et al.*, 1998; Foissac *et al.*, 1999); expression of the soyabean kunitz trypsin inhibitor in rice has also been found to confer resistance to brown plant hopper (Lee *et al.*, 1999). Whilst the effects against aphids have been predominantly in terms of reduced fecundity (i.e. reduced rate of population build-up) and decrease in insect size, for hoppers the effects have been more acute and have also included reduced survival.

Table 1 Transgenic Crops Resistant to Homopteran Pests

Pest	Crop	Gene product	Reference
Myzus persicae	Tobacco	GNA	Hilder et al 1995
	Potato	GNA	Gatehouse et al 1996
	Potato	ConA	Gatehouse et al 1999
Aulacorthum solani	Potato	GNA	Down et al 1996
Macrosiphum euphorbiae	Tomato	Mi1	Vos et al., 1998
Sitobion avenae	Wheat	GNA	Stoger et al., 1999
Aphids	Tobacco	CryIA(ab)/GNA	Wang & Guo, 1999
Nilaparvata lugens	Rice	GNA	Rao et al., 1998
	Rice	GNA	Foissac et al., 1999
	Rice	SKTI	Lee et al., 1999
Nephotettix virescens	Rice	GNA	Foissac et al., 2000
Bemisia tabaci	Tobacco	Manduca serpin	Thomas et al., 1995a
	Cotton	Manduca serpin	Thomas et al., 1995b

STRATEGIES TO IMPROVE LEVELS OF RESISTANCE

Whilst proteins have been identified with varying levels of insecticidal activity towards homopteran insect pests, expression of the respective encoding genes have not given the anticipated levels of crop protection; at best the levels of resistance achieved can only be described as partial. This is probably a consequence of at least two factors. Firstly, the insecticidal activity of the compounds, particularly against aphids, are not sufficiently high; this problem is being addressed both by protein engineering of already available insecticidal proteins, and by the identification of secondary metabolites whose biosynthetic pathways in plants are amenable to modification using plant transformation techniques. The second, and probably more pertinent problem, relates to protein trafficking i.e. in obtaining good levels of the transgene product within the desired tissues.

In order to achieve acceptable levels of pest control via recombinant DNA technology, it is important that the transgene product is adequately expressed in tissues from where that particular target pest feeds. Since Homoptera are predominantly phloem feeders, it is thus necessary that the product is secreted into the phloem sap. Although expression in the phloem has been obtained using constitutive promoters, it was considered desirable for homopteran control to selectively express the insecticidal proteins in the phloem, so as to maximize expression of the insecticidal protein at the site of attack while minimizing it elsewhere in the plant. In an attempt to achieve this goal, the promoter region from the rice sucrose synthase-1 gene (RSs1) was isolated and fused with the coding sequences for β-glucuronidase (GUS) and GNA for expression in tobacco. Subsequent immunological assays demonstrated specific expression in the phloem tissues, with GNA detected in the honeydew from *M. persicae* feeding from theses plants (Shi *et al.*, 1994). More recent studies however, have failed to detected the presence of GNA in the honeydrew from either BPH or GLH fed on transgenic rice plants irrespective of whether GNA was expressed from the constitutive promoter (Ubi) or the phloem mspecific promoter (RSs1), despite it being detected in honeydrew from insects fed artificial diet containing GNA (Foissac *et al.*, 2000). These results suggest that, in this instance at least, the protein is not being delivered efficiently to the insect, and it is possible that many of the problems that have been encountered in attempting to produce crops with high and commercially viable levels of resistance to sap sucking pests have been due, at least in part, to inefficient trafficking of the transgene products. In order to address these specific problems a new generation of promoters are being isolated.

EFFECTS OF TRANSGENE EXPRESSION ON APHID PREDATORS AND PARASITOIDS

If transgenic insect-resistant crops are to play a useful role in crop protection, it is apparent that they must be compatible with other strategies for pest control, not least with biological control where natural enemies of insect pests are utilised. Indeed, the recommended practices for deploying transgenic crops are all based on integrated pest management (IPM). Irrespective as to whether aphids are susceptible to the transgene products expressed, or not, if they are able to take up theses proteins the possibility then exists for these compounds to be passed on to the third trophic level i.e. to predators and parasitoids of the "dosed" aphids. Much attention is now beginning to be focused on the environmental impact of deploying transgenic insect-resistant crops in terms of their effects on predators and parasitoids. Birch *et al.* (1999) recently reported that although aphids colonising GNA expressing potato plants were not acutely toxic to ladybird adults, fecundity, egg viability and longevity were significantly decreased, but that these effects were reversible. However, there was no demonstration of cause and effect and these observations may have been pleiotrophic, due to the genetic transformation process itself (Schuler *et al.*, 1999). Subsequently in a series of experiments with dosed aphids containing quantified levels of GNA, no acute toxicity was observed in ladybird larvae feeding on those aphids (Down *et al.*, 2000); similar studies with adult ladybirds also failed to demonstrate toxicity (unpublished). Interestingly, parasitism of the tomato moth *Lacanobia oleracea* by the ectoparasitoid *Eulophus pennicornis* was improved in the presence of GNA (Bell et al., 1999). Clearly there are insufficient case studies available to make predictive models but results published to date are encouraging.

although much more work is still required. None the less, deployment of transgenic insect-resistant crops has a significantly lower deleterious impact on beneficial insects compared to current agricultural practices i.e. pesticide application.

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Modelling of aphid pests: an aid to rational management

D Morgan
Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK

ABSTRACT

The role of computer model as aids to support the development of rational management of aphid pests is discussed. Two examples of innovative approaches are given to demonstrate the use of models to improve understanding of the complex dynamics and behaviour of aphids, and to determine practical management decisions on the need for and timing of pesticide applications. The first example examines the temporal and spatial dynamics of the grain aphid, *Sitobion avenae*, and the bird cherry-oat aphid, *Rhopalosiphum padi*, in cereal fields, while the second forecasts outbreaks of the pea aphid, *Acyrthosiphon pisum*, in the UK and predicts optimal dates for pesticide applications.

INTRODUCTION

Aphids are prolific pests of crops throughout the world causing damage directly by sucking plant sap, indirectly by spreading viruses, or by facilitating the growth of sooty moulds on their honeydew. Their ability to adapt to and exploit new and established niches result in them being a constant threat to agriculture and although farmers and growers have been combating aphids for over 400 years since Hill (1568) described 'grene fles' attacking horticultural plants, their status as pests is as high as ever and research and development into more effective and rational management options continues. Fundamental to the implementation of any rational aphid management strategy is a thorough understanding of the biology of the target species and a forecast of potential pest populations (Solomon & Morgan, 1994), without which any management strategy is likely to be sub-optimal.

Recent and continuing developments in computer modelling techniques have contributed to improvements in our understanding of insect biology (Hanson *et al.*, 1996) and underpinned practical management support systems (Morgan *et al.*, 1998). Models represent an efficient approach in which complex biological data can be assimilated and synthesised into comprehensible formats that allow researchers to investigate the dynamics and behaviour of insects, and decision-makers to determine prudent pest management options. The objective of this paper is to use two case studies to illustrate how computer modelling techniques can support (a) further understanding of aphid dynamics and behaviour, and (b) appropriate decision-making on the justification and timing of pesticide applications.

METHODS AND MATERIALS

Generic modelling framework

Both of the models described in this paper utilise a generic mathematical framework which simulates insect population dynamics (for a more detailed description see Morgan, 2000). The framework utilises mathematical algorithms to model the development, reproduction, mortality, immigration and emigration of an insect population. Species-specific coefficients are used with the framework to tailor the resulting model to a particular insect population. Moreover, the flexible and modular structure of the framework ensures its applicability to insect ecosystems other than aphids.

Models to aid understanding of the dynamics and behaviour of aphids

The complex interactions between aphids, their hosts and natural enemies, and the environment has meant that it has been difficult and costly to study the many relationships empirically. Computer models have been developed and used successfully to further our understanding of the mechanisms and dynamics of insect ecosystems (Ruesink, 1976). Continuing developments in modelling techniques coupled with a wider availability of more sophisticated computing resources has meant that recent systems have contained more biological realism (Morgan & Morse, 1996).

An example of a system used to examine the population development of aphids is a model that simulates the within-field dynamics and behaviour of the grain aphid, *Sitobion avenae*, and the bird cherry-oat aphid, *Rhopalosiphum padi* (Morgan & Morse, 1996). The generic modelling framework was utilised and additional stochastic algorithms were incorporated to simulate the within-field dispersal of apterous aphids and the inter-relationships with cereal crops, natural enemies and the environment. Results from the model were validated against field results of aphid populations, and a sensitivity analysis was undertaken, whereby small changes were made to each algorithm. The resulting predictions from the sensitivity analysis were compared to investigate the relative contribution that various population processes make to the overall dynamics of the pest.

Models to aid practical aphid management decisions

Simulation models have been used to forecast potential pest outbreaks and to compare available control options so that suitable management strategies can be derived (Solomon & Morgan, 1994). Although in the past there has been some question regarding the use of mechanistic models to forecast pest outbreaks, approaches utilising historical records have made these criticisms redundant (Rabbinge & Rijisdijk, 1983).

An example of a system used to support practical aphid decision-making is a model that predicted outbreaks of the pea aphid, *Acyrthosiphon pisum*. This pest causes significant damage to crops but the timing and extent of the damage is highly variable, differing between sites and between years. The generic modelling framework was utilised to provide forecasts of pea aphid outbreaks and to predict when aphids were likely to exceed action thresholds so that farmers/growers could improve timing of their pesticide applications.

RESULTS

Models to aid understanding of the dynamics and behaviour of aphids

Analysis of the predicted within-field spatial distribution of cereal aphids indicated that early in the season discrete patches were evident but that as population densities increased these became less distinct and that considerable overlapping with neighbouring foci was apparent after 15 days (Figure 1). These spatially-explicit predictions were similar to the dynamics of within-field distributions for aphids found by Winder *et al* (2000) in cereal crops in the UK. Further experimentation with the model was done to compare the effect of small changes in population processes on model predictions. Results indicated that small changes in aphid mortality could have highly significant effects on predicted pest densities: increasing aphid mortality by as little as 5% reduced total aphid density by as much as over 4 fold.

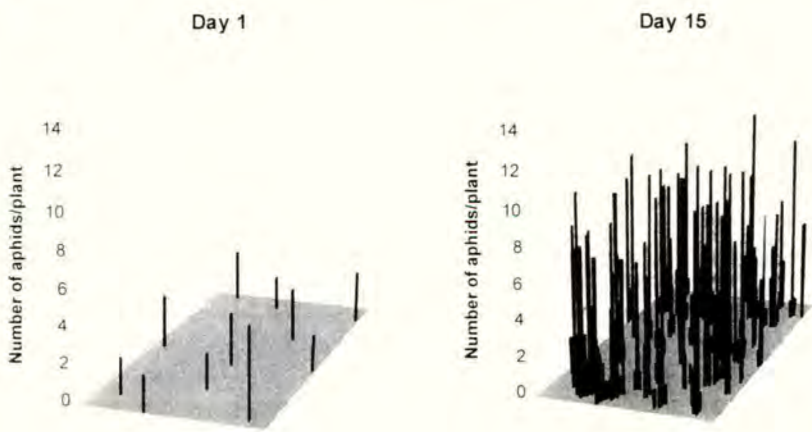

Figure 1. Within-field predictions of the distribution of *S. avenae* populations after 1 and 15 days.

Further investigations examined the effect of within-field distribution of aphids on the effects of pest mortality. In one study it was assumed that an agronomic practice, such as the manipulation of the field margins to enhance aphid control by natural enemies, would be introduced alongside one of the edges of the field and that as a result all aphids adjacent to the boundary would die but that the effect would decline linearly with distance such that an aphid positioned further than a distance of 25% of the width of the field away from the edge would not experience any further mortality. Spatially-explicit simulation results of the standard and increased mortality scenarios are given in Figure 2. Results indicated that even after 15 days the increased likelihood of aphid mortality along only one of the field margins would reduce total aphid populations by between 15-24%.

Models to aid practical aphid management decisions

Forecasts of aphid outbreaks were compared against observed populations at several sites across the major UK pea growing regions over several years. The model predicted accurately the observed development of aphid populations: pest densities remained relatively low during the early part of the season but when environmental conditions and the age-distribution of the resident population were particularly favourable rapid increase in populations occurred (Figure 3). The model was especially accurate at predicting when pest densities were likely to exceed spray action threshold levels and thus when farmers/growers needed to apply insecticides (Figure 3). Consequently the model was integrated with an intuitive computerised delivery system, known as PAM (Pea Aphid Model), to facilitate use by farmers and crop consultants in the UK and has been available commercially since 1998.

Figure 2. Within-field predictions of the distribution of *S. avenae* populations after 15 days with standard mortality and enhanced mortality along the right-hand field margin

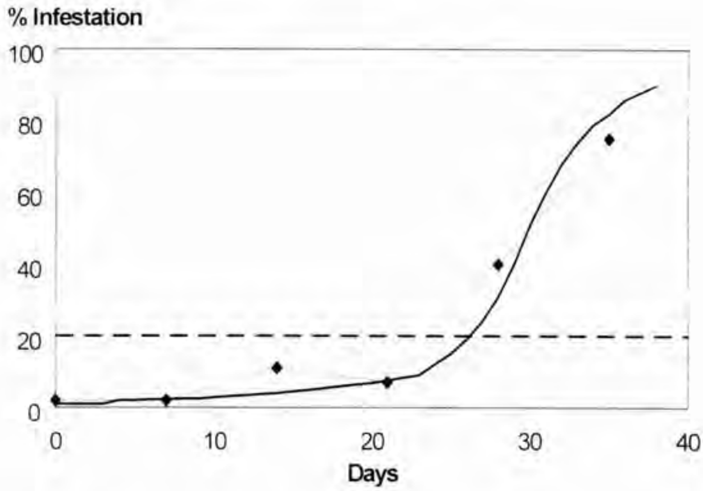

Figure 3. Comparison of observed and predicted percentage shoots infested by pea aphids 1996 (observed ◆·····◆·····◆, predicted ——— and action threshold — — —)

DISCUSSION

The development of computer models to aid rational aphid management is not new: models have been built and used successfully as part of management approaches against many pests in several countries (for example, Gutierrez *et al.*, 1974). Models can provide an efficient mechanism for organising and analysing complex biological data into formats that will allow researchers to experiment with ecosystems, and farmers and crop consultants to rationalise their pest management strategies (Dewar & Carter, 1984). The two case studies presented in this paper indicate some of the roles models can play for both requirements: the cereal aphid model demonstrated how mathematical systems can examine the complex interactions involved in the temporal and spatial dynamics of insects, while the pea aphid system demonstrated how models can produce practical forecasts of when pest populations were likely to need control with insecticides. The use of computer models looks set to become more important and valuable as the amount, number of sources and complexity of information increases, and formulating and interpreting these data to improve our understanding of pests and determine best practice for their management becomes more difficult. Moreover, with the continued increase in the power of computers it will be possible to develop models with greater biological realism with commensurate improvement in accuracy and reliability (Morgan, 2000).

In summary both models presented in this paper have proven to be valuable and powerful tools that aid the achievement of their respective objections of research investigations and practical management support. Although the two examples focus on aphid pests they are both based on a generic modelling framework that has been used successfully to simulate the dynamics of other invertebrate pests (for example Head & Morgan, 1996).

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