SESSION 5A

NEW COMPOUNDS, FORMULATIONS AND USES – INSECTICIDES

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Session Organiser	Mr C Furk *Pesticides Safety Directorate, York*
Papers	5A-1 to 5A-7

DPX-MP062: A NOVEL BROAD-SPECTRUM, ENVIRONMENTALLY SOFT, INSECT CONTROL COMPOUND

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ABSTRACT

DPX-MP062 [Indeno [1, 2-e] [1, 3, 4] oxadiazine-4a (3H)-carboxylic acid, 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl) [(4-trifluoromethoxy) phenyl] amino] carbonyl]-, methyl ester] is a highly efficacious new insect control compound demonstrating broad spectrum control of lepidoptera target pests at rates of 12.5-70 g a.i./ha. Field studies, conducted over several years around the world, demonstrate that DPX-MP062 is highly effective in controlling populations of *Heliothis, Helicoverpa, Spodoptera, Plutella, Trichoplusia, Lobesia, Cydia* and other lepidopteran target pests in various crops such as cotton, vegetable and fruit. The product demonstrates good efficacy on target insect pests while preserving beneficial insects and mites. DPX-MP062 is an enriched active isomer (75% DPX-KN128 insecticidal component). Most of the data here have been developed on the racemic material DPX-JW062.

DPX-MP062 has a very favorable environmental profile with a relatively short half life, low environmental loading, and large margins of safety to mammalian, avian, aquatic, and non-target organisms. DPX-MP062 has a novel mode-of-action, resulting in lack of cross resistance to standard insect control compounds such as pyrethroids, O.P.s, and carbamates and consequently will fit well into resistance management programs, and integrated control situations.

INTRODUCTION

The future of agriculture in a modern society inundated with environmental and toxicological concerns is dependent on the discovery of novel solutions to insect control. Although a number of recent products have been introduced, many suffer from disadvantages in bioefficacy (eg. narrow spectrum, high use rates) or pose threats to the environment (eg. impact on beneficials, impact on aquatic systems). This leaves a clear need of growers for effective low use rate, novel mode-of-action, environmentally sound products that fit into current and future IPM programs. DPX-MP062 insect control agent is the result of an extensive discovery and development program within DuPont to address these specific areas of concern and create a product for the future that meets many of the standards established by both growers and environmental/ regulatory agencies.

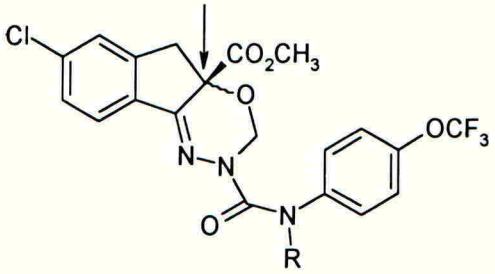
CHEMICAL AND PHYSICAL PROPERTIES

DuPont is currently developing two insecticidal compounds, both of which contain the same active ingredient; these are referred to as DPX-JW062 and DPX-MP062. Both DPX-JW062 and DPX-MP062 contain two optically active isomers, only one of which is insecticidally active. These optical isomers are called DPX-KN128 and DPX-KN127. DPX-KN128 is the insecticidally active isomer and the active ingredient which is common to both DPX-JW062 and DPX-MP062. DPX-KN127 has no insecticidal activity and from a toxicological and environmental perspective can be considered an inert. The chief difference then between DPX-JW062 and DPX-MP062 is in the level of DPX-KN128 and DPX-KN127 they contain. Table 1 describes the different isomer ratios in each compound.

Table 1. Isomer ratios for DPX-JW062 and DPX-MP062.

Compound code	Isomer code	Ratio of isomers (%)
DPX-JW062	DPX-KN128	50
	DPX-KN127	50
DPX-MP062	DPX-KN128	75
	DPX-KN127	25

Structure of DPX-MP062, some physico-chemical properties



R = $COCH_3$ = MP062

R- = DPX-KN127, THE INACTIVE ISOMER

S- = DPX-KN128, THE ACTIVE ISOMER

➔ = Site of the chiral carbon

Chemical Name: Indeno [1,2-e] [1,3,4] oxadiazine-4a (3H)-carboxylic acid, 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-trifluoromethoxy) phenyl] amino]carbonyl]-, methyl ester

CAS Registry No. 144171-61-9

1.	Molecular weight:	527.87 g/mole
2.	Melting point (for solids only):	140-141° C
3.	Solubility (in water, organic solvents, lipids and fats):	Water <0.5 mg/l 1-Octanol 480 mg/l Methanol 390 mg/l Acetonitrile 76000 mg/l Acetone 140000 mg/l
4.	Partition coefficient in octanol/water:	Approx. 40000
5.	Vapour Pressure:	Less than 10^{-5} Pascals at 20-25° C

Physical chemical properties cited here refer to the racemic compound (DPX-JW062)

MAMMALIAN TOXICOLOGY

Technical (based on DPX-JW062)

Acute oral LC50, (rat):	> 5000 mg/kg (EPA Tox. Cat. IV)
Acute dermal LD50, (rabbit):	> 2000 mg/kg (EPA Tox. Cat. III)
Acute inhalation LC50, (rat):	> 2 mg/l (EPA Tox. Cat. III)
Eye, skin irritation, (rabbit):	None
Dermal sensitization, (guinea pig):	No evidence
Ames Test:	Negative

ENVIRONMENTAL SAFETY

Avian toxicity (based on DPX-JW062)

Bobwhite quail and Mallard duck, acute oral LD_{50}: > 2250 mg/kg
Bobwhite quail and Mallard duck, 5-day dietary LC_{50}: > 5620 mg/kg diet

Aquatic toxicology

Bluegill sunfish 96 hr. LC_{50}: > 1.0 mg/l
Rainbow trout 96 hr. LC_{50}: > 0.5 mg/l

Environmental Fate

Soil half-life: 4-5 days tama silt loam soil
Aqueous Hydrolysis: pH 5 > 30 days, pH 7 ~ 30 days, pH 9 ~ 2 days
Aquatic Photolysis: 1-2 days at pH 5.0

Beneficial Arthropod Evaluations

Following 4-6 applications of 30-50 g a.i./ha DPX-KN128, little or no adverse effects were reported on the parasitic wasp *Aphidius rhopalosiphi*, predatory mite *Typhlodromus pyri*, ground dwelling predator *Aleochara bilineata* and aphid predator *Episyrphus balteatus* (Mead-Briggs *et al.*, 1996).

BIOLOGICAL PROPERTIES - FIELD STUDIES

Table 2. Control of *Lobesia botrana* on vine in Europe (1995)

Treatment	Rate g a.i./ha	France		Italy		Germany
		L*	D**	L*	D**	L*
DPX-KN128	30	89	87	88	85	96
DPX-KN128	37.5	98	91	92	96	97
DPX-KN128	45	97	91	96	97	-
chlorpyrifos	285	84	82	48	71	-
l-cyhalothrin	17.5	79	64	-	-	-
m-parathion	120	-	-	-	-	72
Untreated		(323)	(728)	(33)	(52)	(89)

* L = % control (based on larvae/100 bunches)
** D = % reduction (based on holes/100 bunches)
( ) = No. larvae or holes/100 bunches

Table 3. Control of *Plutella xylostella* and *Trichoplusia ni* on head cabbage (Florida, USA, 1996)

Treatment	Rate g a.i./ha	% Control
DPX-KN128	25	98
DPX-KN128	50	96
l-cyhalothrin	40	90
tebufenozid	50	50
tebufenozid	100	69
Untreated	-	(240)

( ) = No. of larvae/100 plants

Table 4. Control of pests on tomato and sweet pepper (1995)

Treatment	Rate g a.i./ha	Spain* (pepper)	Spain** (tomato)	Australia*** (tomato)	USA**** (tomato)
DPX-KN128	25	96	96	-	85
DPX-KN128	37.5	97	100	89	-
DPX-KN128	50	-	-	95	91
DPX-KN128	62.5	-	-	99	100
deltamethrin	12.5	93	48	-	-
sulprofos	720	-	-	95	-
chlorfenapyr	225	-	-	-	67
Untreated		(28)	(68)		

* *Spodoptera exigua* ** *Plusia gamma* *** *Helicoverpa armigera* ****S. eridania*
( ) = No. of larvae

Table 5. Control of various pests on apple (Pennsylvania, USA, 1995)

Treatment	Interval (days)	Rate g a.i./ha	% Clean Fruit (at harvest)	% infest *Cydia pomonella*	% control *Typhlocyba pomaria*	% control *Phyllonorycter blancardella*
DPX-KN128	10	50	83	4	68	58
DPX-KN128	10	75	88	4	69	46
DPX-KN128	14	50	87	6	66	56
DPX-KN128 + azinphos-methyl	14	75 + 250	93	1	70	47
azinphos-methyl	14	500	87	2	66	47
Untreated	-	-	48	29	0	0

Table 6. Control of various pests on apples in Europe (1995)

Treatment	Rate (g a.i./hl)	France*	Germany**	Spain*	Hungary*
DPX-KN128	2.5	-	71	-	-
DPX-KN128	3.8	-	100	-	-
DPX-KN128	4.0	65	-	80	92
DPX-KN128	5.0	88	-	100	98
azinphos-methyl	43.7	88	-	100	94
phosalone	60	82	46		95
Untreated		17	12	3	15

* % control of *Cydia pomonella*

** % control of *Adoxophyes orana, Archips podana*

Table 7. Control of cotton pests (1995)

Treatment	Rate g a.i./ha	USA*	Spain**
DPX-KN128	38	93	89
DPX-KN128	50	90	91
DPX-KN128	63	-	100
DPX-KN128	75	100	-
thiodicarb	840	93	-
l-cyholothrin	34	83	-
deltamethrin	19	-	60
Untreated	-	(29)	(12)

* % control of *H. virescens* (based on No. larvae/100 terminals)

** % control of *Helicoverpa armigera* (based on No. larvae/100 fruit)

( ) = No. larvae/100 terminals

MODE OF ACTION

DPX-KN128 offers a totally novel mode-of-action compared to other insect control products. Biochemical studies have demonstrated that DPX-KN128 (Wing, personal commun.) and related chemistry (Salgado, 1990) blocks sodium channels in nerve cells. The blockage of these sodium channels in insects leads to poor coordination, paralysis and ultimately death of the target insect.

The routes of entry into insects is via both contact and ingestion. Insect behaviour is rapidly altered following exposure to a toxic dose of DPX-KN128, resulting in a rapid cessation of feeding and consequently excellent plant protection of the target crop. Extensive laboratory and field studies on strains resistant to a broad range of commercially available products demonstrate a lack of cross resistance to DPX-KN128, thus offering a valuable tool for IPM and resistance management programs.

SUMMARY

DPX-MP062 is a novel insect control agent containing the active ingredient DPX-KN128. Tests on a range of crops and pests worldwide have shown outstanding larval control in the range 12.5-70 g a.i./ha. The low toxicity to non-target organisms and short persistence in the environment indicate that DPX-KN128 is surprisingly environmentally benign for such an effective control agent. The novel mode of action of this molecule raises the probability of effective control within the framework of IPM and resistance management strategies.

ACKNOWLEDGEMENTS

Product development involves extensive teamwork across disciplines. We would like to express our graditude to all our colleagues who have contributed to our understanding of the value of DPX-MP062.

REFERENCES

Mead-Briggs, M; Bakker, F. M.; Grove, A.J.; Primiani, M. M. (1996). Evaluating the effects of multiple-application plant protection products on beneficial arthropods by means of extended laboratory tests: case studies with predatory mites and hoverfiles, and the insecticides DPX-JW062 and DPX-MP062. Brighton Crop Protection Conference - Pests and Diseases 1996 (In Press)

Salgado, V. L. (1990). Mode of Action of Insecticidal Dihydropyrazoles: Selective Block of Impulse Generation in Sensory Nerves. Pesticide Science, **28**, 389-411.

A NEW BROAD-SPECTRUM AND HIGHLY ACTIVE PYRETHROID - ZXI 8901

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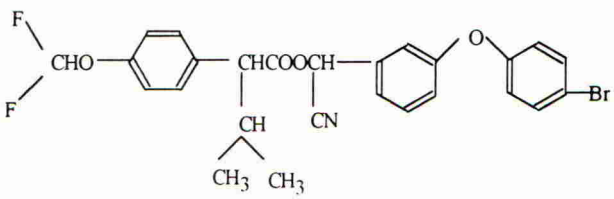
ABSTRACT

ZXi 8901 is a new broad-spectrum and highly active pyrethroid invented in our research laboratory. ZXI 8901 is a highly active compound for the control of more than 30 kinds of insects and spider mites, and has low toxicity to animals and bees. The synthesis and toxicity of ZXI 8901, and its effectiveness for the control of different kinds of insects and spider mites in the greenhouse and the field are discussed.

INTRODUCTION

After the invention of the pyrethroids with the characteristic of stability in light and oxygen, many new kinds of pyrethroid insecticides have been produced and used in agriculture. In the structure of some pyrethroids, the cyclopropane radical has been substituted and consequentely the synthetic method has been simplified. In some cases, the introduction of a fluorine atom to the structure of the molecule has extended the activity to include the control of spider mites e.g. bifenthrin, cyfluthrin, acrinthrin, cyhalothrin, flucythrinate etc. In the above pyrethroids, except flucythrinate, the synthesis of all the compounds is very complicated. However flucythrinate can not be used as an acaricide and its toxicity for the user is higher than the others, e.g. its emulsifible concentrate is a strong irritant to human beings. In order to find new pyrethroids with high activity for insects and spider mites, and to be easy to synthesize and at low cost, some new derivatives have been synthesized and screened for their activity by using flucythrinate as a model for the basic structure. In 1989, a new pyrethroid that had not been published in patents and literature at that time in the world was invented in our laboratory.

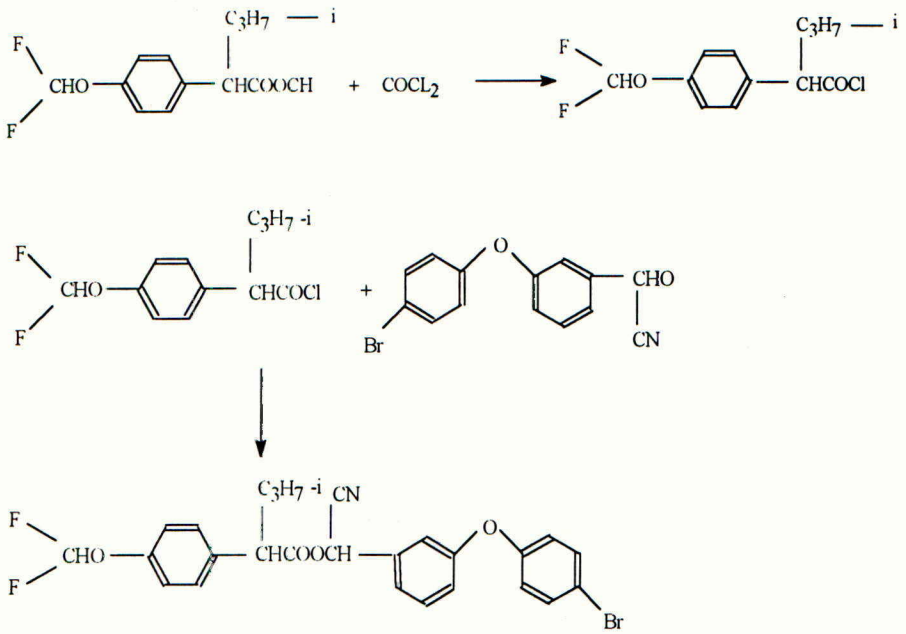
The chemical structure of ZXI 8901 is as follows:



After five years of large scale field trials, and the evaluation of the safety and environmental effects, ZXI 8901 has been proved to be a new pyreroid with low toxicity, high activity and a broad spectrum of control for both insects and spider mites.

SYNTHESIS OF ZXI 8901:

ZXI 8901 is synthesized by the following reaction series:



SAFETY EVALUATION

Table 1. Results of the toxicity tests of ZXI 8901

Acute LD50 mg/kg :	
Oral, male rats	>10,000
Oral, female rats	>10,000
Oral, male mice	>12,600
Oral, female mice	>12,600
Dermal, male rats	>20,000
Dermal, female rats	>20,000
Irritant test for rabbits:	
For eyes	no irritant effect
For skin	no irritant effect

Micronucleus test for mice		no effect
Testis chromosome teratogenesis test of mice		no teratogenous effect
Ames test		no effect
Dominant lethal test		no effect
Allergy test for guinea pig		weak allergic substance
Subchronic test for 90 days		minimum effective dosage (mg/kg/day) SD male rats 102.0±32.8 SD female rats 132.1±37.5 maximum no effect dosage (mg/kg/day) SD male rats 19.6±7.9 SD female rats 27.4±8.4
Chronic toxicity test(2 years)		in process
Environmental behaviour data		
Toxicity to fish (*Cyprinus carpio*) LC50	24h	5.49mg/l
	48h	1.99mg/l
	96h	0.48mg/l
Toxicity to water fleas (*Daphnia magna*) LC50	24h	39.1μg/l
	48h	14.3μg/l
Toxicity to algae (*Scenedesmus obliquus*) EC50	24h	0.38mg/l
	48h	0.26mg/l
	96h	0.23mg/l
Toxicity to birds (*Coturnix aponica*) LD50		337mg/kg
Toxicity to honey bees (*Apis mellifera*) LC50		957.7mg/l
Toxicity to silkworms (*Bombyx mori*) LC50 (poisoning of stomach)		0.17mg/kg(leaf of mulberry)
(poisoning of contact)		0.13ug/cm2 (paper)
Toxicity to earth worm (*Eisenia feltida*) LC50		
(red soil)		53.3mg/kg (soil)
(moisture soil)		100.9mg/kg (soil)
(yellowish brown soil)		165.0mg/kg (soil)
Mobility of soil (TLC)		Rf < 0.1
Half life in soil (T1/2)		4.8-8.8 day
Half life of hydrolysis at water (T1/2)	PH 5	15.6 day
	PH 7	8.3 day
	PH 9	4.2 day
Half life of photolysis at solution (T1/2)		
water phase		13.7 min.
petroleum ether phase		9.4 min.
KOW (Log Kow)		4.35

EFFECTIVENESS TESTS

Glasshouse trials

Three kinds of insects were used: bollworm (*Heliothis armigera*) as a representative of the Lepidoptera; Turnip aphid (*Rhopalosiphum pseudobrassicae*) as an aphid; *Tetranychus telarius* and *Tetranychus viennensis* as spider mites. Tests were made using a Potter Tower. The results are as Table 2.

Table 2 Results of glasshouse trials to test the effectiveness of ZX 18901 (mg/l)

	Tetranychus telarius		*Tetranychus viennensis*		*Rhopalosiphum pseudobrassicae*		*Heliothis armigera*	
Product	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Zx 8901	13.88	70.78	8.76	109.6	0.339	10.03	0.04	0.39
Dicofol	16.3	138.2			0.53	10.13	10.06	0.6
Amitraz	58.96	836.5	43.29	638.4				
Cyhalothrin	79.69	258.7	4.5	51.76	0.04	2.58		
					0.02	0.4		
Deltamethrin					0.05	370.0		
Fenvalerate					0.85	48.1	0.02	0.17
					0.54	70.7	0.01	0.14

From the above results, we can find that ZXI 8901 is very effective for the control of spider mites. Its activity against *T. telarius* is 4 times of that of amitraz and 4.95 times of that of cyhalothrin. Against *T. viennensis* , the activity of ZXI 8901 is only half of that of cyhalothrin, but is 5 times of that of amitraz and 12.4 times more active than dicofol. For turnip aphid, ZXI 8901 is less active than that of cyhalothrin and deltamethrin, but more active than fenvalerate. For bollworm, the activity of ZXI 8901 is the same as fenvalerate.

Field trials

From 1990 to 1994, field trials of ZXI 8901 have been carried out. The tests were done in more than 11 provinces and cities, such as Shandong, Sichuan, Hebei, Jiangsu, Jiangxi, Fujian, Shanxi, Beijing, Hubei, Hailongjiang and Xinjiang. The crops and insects tested are as follows:

Cereals: Armyworm; *Toxoptera graminium; Macrosiphum granarium.*
Cotton: Bollworm; *Tetranychus telarius; Pectinophora gossypiella; Aphis gossypii.*
Vegetables: mites of red cowpea; mites of pepper; cabbage worm; mites of egg plant; *Plutella maculipennis; Pieris rapae; Barathra brassicae; Brevicoryne brassicae.*

Fruits:	*Panonychus citri; Carposina sasakii; Panonychus ulmi; Aphis citrella; Phyllocnistis citrella; Psylla pyrisuga*; mite of water melon.
Tea:	*Empoasca formosana; Ectropis obligua.*
Soyabean:	*Grapholitha glycinivorella; Aphis glycines.*
Other:	Looper of tung tree; mite of bees; *Laphygma exigna; Agrotis tokionis.*

Results show that:

1. ZXI 8901 is very toxic to spider mites and very effective in the field for the control of the 10 kinds of spider mites tested. In comparison with the other pyrethroids for the control of mites, ZXI 8901 is as active or more active than fenpropathrin and cyhalothrin. ZXI 8901 is better than amitraz and dicofol in the field tests, especially for the control of tea mite.

2. The control spectrum of ZXI 8901 in the field tests is broad. In addition to its high activity to spider mites, ZXI 8901 is very effetive for the control of Lepidoptera spp. and aphids. The results from the field trials in 11 different provinces and cities, proved that ZXI 8901 is a very effective insecticide and acaricide with broad control spectrum.

3. ZXI 8901 is more effective than cyhalothrin, fenpropathrin and cypermethrin for the control of borers, such as *C. sasakii*. Control was 94.1-96.0% when the insecticide was used at 60g a.i./ha and it was 73.4% when used at 30 g a.i./ha.

4. ZXI 8901 is also better for the control of eggs. ZXI 8901 gave 73.8% control of the eggs of *C. sasakii* used as 15 g a.i./ha. This result was markedly better than flucythrinate at the same concentration.

5. Results also demonstated long residual effects. From the results of field trials for the control of fruits pests (such as *T. viennensis* and *P. ulmi*), control was 60.54% after 14 days sprayed with 60g a.i./ha. The effectivity after 15 days sprayed with 120g ZXI 8901/ha, for *P. rapae* was 95.20%; for *B. brassicae* was 84%; for *E. obligua* was 86.5%; for Looper of tung tree was 90%; for *P. citrella* was 73.7% after 25 days of the spray. The above results show that ZXI 8901 is a pyrethroid with good residual effects.

6. A large scale field trial was carried out in Xinjiang province. The total area of the trial was 83.7 ha consisting of 15.3 ha of sugar beet, 0.67 ha of water melon, 1 ha of chinese cabbage and 71.33 ha of cotton (Table 3).

7. ZXI 8901 has low toxicity to bees and is very effective for the control of mites of bees. The LD50 for the bee is 975.7 mg/l. The LD50 for the mite of bees is 1.774 mg/l.

Table 3 Results of ZXI 8901 used in a large scale field trial in Xinjiang province

Insect	Concentration of ZXI 8901 (g a.i./ha)	DAT	Control (%)	Standard insecticide	Control (%)
A	60	10	98.29-99.16	Monocrotophos	93.21-96.34
B	60	15	99.57	Monocrotophos	98.16
C	60	10	95.0	Monocrotophos	85.8
C	60	10	93.33	Monocrotophos	57.1
				Cypermethrin	92.6
D	30	10	96.3	Fenbutatin oxide	99.32
E	45	15	99.28	Cypermethrin	97.38
F	45	10	98.0	Monocrotophos	90.67
F	30	10	99.8	Monocrotophos	94.47
G	45	10	99.48	Monocrotophos	95.08
G	30	10	100.0	Monocrotophos	98.41

A=Mite of cotton leaf, B=*Aphis gossipii* , C=*Heliothis armigera*, D=Mite of water melon, E=Aphids of water melon, F=*Agrotis tokionis* , G=Noctuid of trefoil

DISCUSSION

The above results shows that ZXI 8901 is a very effective, and broad spectrum insecticide and acaricide new pyrethroid with low toxicity. ZXI 8901 is very effective for the control of harmful insects and spider mites, and is very safe for human beings and bees. ZXI 8901 can be used in many kinds of crops, especially economic and food crops. We believe that ZXI 8901 will be a good insecticide and acaricide for protection in agriculture.

REFERENCES

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A NEW MICROBIAL INSECTICIDE, *PAECILOMYCES FUMOSOROSEUS* STRAIN APOPKA 97, FOR THE CONTROL OF THE GREENHOUSE WHITEFLY

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ABSTRACT

PFR-97 contains a proprietary strain of the entomopathogenic fungus *Paecilomyces fumosoroseus* strain Apopka 97. This new microbial insecticide provides excellent control of the greenhouse whitefly, *Trialeurodes vaporariorum*, on greenhouse vegetables, e.g. cucumber. The development of PFR-97 as a biological control product is discussed in view of its application as a selective IPM tool.

INTRODUCTION

Paecilomyces fumosoroseus is a naturally occurring fungus in most countries of the world. Like most entomopathogenic fungi *P. fumosoroseus* may be found in various soil types at very low densities. The fungus has frequently been isolated from infected insects throughout the world (Table 1).

P. fumosoroseus strain Apopka 97 (PFR 97) was isolated in 1986 from the mealybug *Phenococcus solani* on *Gynura* in a conservatory in Apopka, Florida, by Prof. Dr. Lance Osborne, Agriculture Research Center, Florida University. The patented strain was licenced to Thermo Trilogy Corporation (formarly W.R. GRACE & Co.-Conn, GRACE Biopesticide Division) who developed the production and formulation. Further field development of *P. fumosoroseus* strain Apopka 97 has been done by Biobest, Belgium, for the control of whitefly in greenhouse vegetables, ornamentals and small fruits in Europe. This fungus will be used to support the biological control of whiteflies with the parasitoid *Encarsia formosa* and other natural enemies like the mirid bug, *Macrolophus caliginosus*.

Table 1. Distribution of *Paecilomyces fumosoroseus*.

Country	Isolated/Source	Strain #	Reference
Brazil	*C. rotundus*	ARS 3480	Tigano-Milani *et al.*, 1993
	Soil		Domsch & Gams 1979
	Lagria uilosa	ARS 2216	ARS fungi collection
	Spaethilla sp.	ARS 2956	"
Canada	Soil	IMI 113171	IMI collection,1965
	Gypsy moth egg	CCFC004425	CCFC collection, 1982
Dom.Republic	*Bemisia tabaci*		Colmar-A-Serral, 1993

Country	Isolated/Source	Strain #	Reference
China	*T.vaporariorum*		Fransen,1994
Costa Rica	Hemiptera	ARS 3429	ARS collection,1991
Czech	Termite		Krejzova, 1976
	Soil	1407,1426	Landa *et al.*, 1994
	Soil	1735,1650	"
France	*M. melolontha*	CBS 106.66	CBS collection, 1966
	Calliphora sp.	ARS 1646	ARS collection, 1984
	Thaumatopoea sp.	CBS 337.52	CBS collection, 1952
	Musca domestica	ARS 1867	Tigano-Milani *et al.*, 1995
	P. luteloa	ARS 1506	"
	Scotiz seqetum	No. 39	Rodriguez Rueda *et al.*, 1980
	S. aphodii	CBS 107.10	ARS collection,1910
	M. autumnalis	ARS 1626	", 1984
Finland	Soil		Vanninen *et al.*, 1989
Germany	*T. pityoc*	CBS 309.59	Samson 1974
	Soil		Zimmerman 1986
Ghana	*Anachetus* sp.	CBS 721.73d	Samson 1974
	Lepidoptera	CBS 721.73a	CBS collection, 1973
	Mealy bug	CBS 721.73b	"
	Hymenoptera sp.	CBS 721.73c	
Greece	*B. tabaci*		Lacey *et al.*, 1993
Holland	Agriculture	CBS 101.73	Samson 1974
	Air	CBS 264.58	"
	Sputum	CBS 339.54	"
India	*B. tabaci*	ARS 3700	Colmar A. Serra 1993
Indonesia	*N. lugens*	ARS 2429	ARS collection 1987
	Larva Diptera	ARS 2425	"
Ireland	Butter	CBS 244.31	CBS collection 1971
Italy	*M. elongctuls*	ARS 1576	ARS collection, 1984
	H. cunea	ARS 1568	"
	Adelphocoris sp.	ARS 1532	"
	T. pitycampa	CBS 309.59	CBS collection, 1958
	S. nonagrioides		Nanni *et al.* 1988
	P. fasciana		"
Japan	Food	CBS 375.70	CBS collection 1970
	Bombyx mori	ARS 989	ARS collection 1995
Mexico	*B. tabaci*	ARS 3313	ARS collection 1995
	D. hyalinata	ARS 3302	ARS collection 1990
Nepal	*B. tabaci*	GC405	Colmar A. Serra 1993
Pakistan	*B. tabaci*	CG 406	Colmar A. Serra 1993
Philippines	Soil	ARS #	ARS collection 1990
	N. lugens	ARS 2198	ARS collection 1985
	P. xylostella	ARS 2749	ARS collection 1989
Poland	Soil		Mietkiewski 1991
Spain	*B. tabaci*		Lacey *et al.*, 1993
South Africa	*B. tabaci*		Lacey *et al.*, 1995

Country	Isolated/Source	Strain #	Reference
Sri Lanaka	Soil	IMI 133019	IMI collection 1968
Switzerland	Apple sawfly		Bolckmans *et al.*, 1995
Trinidad	*Bemisia tabaci*		Hall *et al.* 1994
U.S.A	*B. tabaci*	PFR 97	Osborne *et al.*,1991,1992
	B. tabaci	ARS #	ARS collection
Russia	*L. sticticollis*	CBS 243.31	CBS collection, 1959
Venezuela	*B. tabaci*		Lacey *et al.* 1995

General considerations

For the past eight years researchers in various universities have worked with the Apopka 97 strain on a large number of noxious soil and foliage insects. Biobest N.V. and Thermo Trilogy Co. decided to focus the research effort on the development of the formulated product, PFR-97, trade name PreFeRal, for the control of whiteflies in European greenhouses.

TOXICOLOGY OF PFR-97

Human and animal toxicology

Paecilomyces fumosoroseus strain Apopka 97 cannot grow at temperatures above 32° C which is why this fungus cannot grow at temperatures such as those of the human body. This is clearly indicated by the toxicity tests on mammals (Hartmann *et al.* 1979, Donovan-Peluso *et al.* 1980). Tests conducted with *P. fumosoroseus* strain Apopka 97 indicate that this fungus is not toxic (Table 2). The Ames-test (mutagenicity) was also conducted. This test yielded fully favourable results.

Table 2. Summary of acute toxicology

Acute oral toxicity/Pathogenicity	No toxicity, pathogenicity, or infectivity at 10^6 CFU/animal
Acute dermal toxicity	No toxicity at 10^9 CFU/animal
Acute pulmonary toxicity/Pathogenicity	No toxicity, pathogenicity or infectivity at 10^6 conidia spores/animal
Acute interperitoneal toxicity/Pathogenicity	No toxicity, pathogenicity or infectivity at 10^7 CFU/ml
Primary eye irritation	Practically non-irritating at > 10^7 CFU/animal
Primary dermal irritation	Slight irritant reversible within 72 hours at 10^8 CFU/animal
Dermal sensitization	Not a sensitizer at 10^7 CFU/animal
Hypersensitivity incidents	None reported

Furthermore, tests showed that *P. fumosoroseus* strain Apopka 97 does not produce any known mycotoxins. The mode of action of this fungus on insects is probably merely mechanical and enzymatic.

ENVIRONMENTAL FATE

P. fumosoroseus strain Apopka 97 is harmless to mammals and birds and does not pose any threat to flora and fauna. Tests on birds, bumblebees and different species of beneficial arthropods such as predatory mites, flower bugs, mirids and parasitoids all clearly proved that the fungus is completely safe for the environment (Table 3). The product will be used to support biological control in tunnels and greenhouses (Bolckmans *et al.* 1995). All trials on side-effects on beneficial arthropods were carried out by Biobest N.V., except the *Encarsia* trials which were done by Dr. M. Van de Veire (University of Ghent) (Sterk *et al.* 1995a), and the trials on adult bumblebees, which were done by Dr. Derwael (Governmental Station for Nematology and Entomology of Merelbeke, Belgium) (Sterk *et al.*, 1995b).

Table 3. Toxicity of PFR-97 on beneficial arthropods

Organism	Mortality (%)	IOBC class
Orius laevigatus (Anthocoridae) larvae	0	1
Orius insidiosus (Anthocoridae) larvae	0 to 11	1
Macrolophus caliginosus (Miridae) larvae	7 to 26	1-2
Therodiplosis persicae (Cecidomyiidae) larvae and pupae	32	2
Phytoseiulus persimilis (Phytoseiidae) adults	0 to 40	1-2
Amblyseius degenerans (Phytoseiidae) adults	0 to 25	1
Typhlodromus pyri (Phytoseiidae) mixed population	0	1
Encarsia formosa (Eulophidae) adults	0	1
Encarsia formosa (Eulophidae) reduction in parasitation	10	1
Bombus terrestris (Apidae) Adults Oral toxicity	No effect	
Bombus terrestris (Apidae) Adults Contact toxicity	No effect	
Bombus terrestris (Apidae) Larvae Oral toxicity	No effect	

Class 1 : non-toxic (harmless) Class 2 : slightly toxic
Class 3 : moderately toxic Class 4 : toxic (harmful)

EFFICACY OF PFR-97

Several single treatment trials on the greenhouse whitefly with PFR-97 at different dose rates on cucumber were carried out by Biobest N.V. during the last three years (Table 4).

Table 4. Percent mortality of greenhouse whitefly using *Paecilomyces fumosoroseus* strain Apopka 97

COMPOUND	Dose rate % compound	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
PFR-97	0.100	92.8	87.1	96.6	87.1	99.8
Applaud	0.030	93.9		99.0		100.0
Admiral	0.025	77.1	95.9	92.5	95.9	96.6

CONCLUSION

It is clear that PFR-97 has a very high efficacy against larvae of the greenhouse whitefly, *Trialeurodes vaporariorum*. It is completely safe to mammals. The fungus has also no undesirable ecological side-effects.
This naturally occuring fungus will be used to support biological control in greenhouses.

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Sterk, G ; Bolckmans, K; De Jonghe, R; De Wael, L;Vermeulen, J (1995 b) Side-effects of the microbial insecticide PreFeRal WG (*Paecilomyces fumosoroseus*, strain Apopka 97), on *Bombus terrestris*. *Mededelingen Faculteit Landbouwkundige en Toegepaste Wetenschappen Universiteit Gent*, **60**, 713-717.

IMIDATE INSECTICIDES: A NEW CLASS OF BROAD SPECTRUM INSECTICIDES

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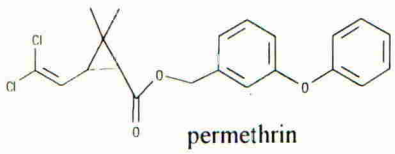
ABSTRACT

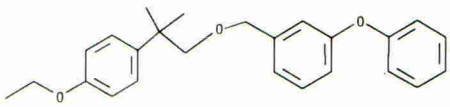
A new class of imidate insecticides have been discovered which have significant activity against *Heliothis virescens, Musca domestica, Trichoplusia ni* and a wide variety of other economically important pests. These compounds also display favourable environmental properties such as short half life and low toxicity to both mammals and fish. This paper examines the rationale that led to the discovery of these new compounds, structure activity relationships in the series - including strategies which led to improved activity, and their environmental and toxicological profile. A particularly effective strategy for optimisation of the activity of the series was based on blocking metabolism resulting in higher overall activity. Activity against *Homoptera* species was also optimised with particular emphasis on *Nephotettix cincticeps*, a major pest in rice. Mode of action studies have shown these insecticides are likely to share their mode of action with pyrethroids

INTRODUCTION

In 1983 it was reported that membrane receptors in the nervous systems of *Musca domestica* and *Heliothis virescens*, critical for the insecticidal activity of DDT and pyrethroids, appeared to bind both of these classes of insecticides at the same receptor but not at precisely the same site (Chang & Plapp, 1983a,b). These reports led to our proposal that new compounds combining some structural features from DDT and a pyrethroid might also be able to bind to this receptor and show insecticidal activity. In addition, it was desired to give the new compounds structural features which would make them less persistent in the environment than DDT but more stable to enzymatic hydrolysis, and thereby longer lasting, than pyrethroid esters such as permethrin. One way to accomplish the latter objective is to replace the ester functionality of pyrethroids by a more hydrolytically stable linkage such as an imidate ester or an amidine. A precedent for replacing the ester linkage of pyrethroids with an ether and maintaining activity had already been established with the insecticide etofenprox.

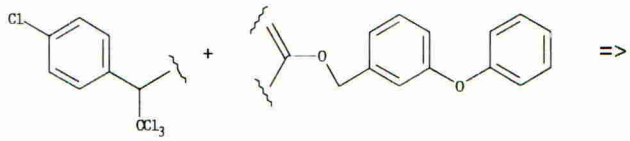


permethrin



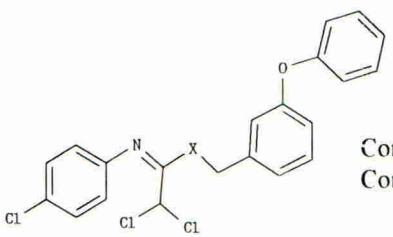
etofenprox

These considerations led to the synthesis of an imidate ester (Compound 1) and an amidine analogue (Compound 2). These compounds have a *p*-chlorophenyl and a polychloromethyl group taken from the DDT structure, a *m*-phenoxy benzyl moiety from the pyrethroid insecticides, and a central linkage more stable than an ester. Both compounds showed sufficient activity against a range of insects to warrant further investigation and optimisation. These lead compounds were therefore discovered using biorational principles.



From DDT

From permethrin

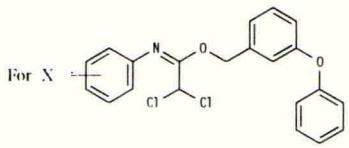


Compound 1, X=O
Compound 2, X=NH

INITIAL STRUCTURE ACTIVITY STUDIES

Preliminary structure activity studies revealed far more scope for improvement of activity through further synthesis with imidates than with amidine analogues, such as Compound 2. Therefore, the early structure activity studies focused on imidates and variation of substituents for the N-aryl ring and replacements for the dichloromethyl group, based on the presumption that the SAR in the phenoxy benzyl moiety would follow previous studies with ester pyrethroids.

It was found that ortho substituents generally reduced the overall activity of N-aryl imidates while substituents in the meta and para positions were generally acceptable as long as they were not large in size. Data for selection of substituents are displayed graphically in Figure 1 based upon relative effectiveness in controlling *Trichoplusia ni*. These early optimisation studies showed that 3,4 dichlorophenyl, 3 chloro 4 fluorophenyl, 4 trifluoromethoxyphenyl and 3,4 methylenedioxyphenyl gave the best overall activity with the ultimate ranking of these varying from species to species. These general preferred substituent patterns for the N-aryl moiety were found to be maintained during analogue work around the dichloromethyl and phenoxy benzyl moieties.



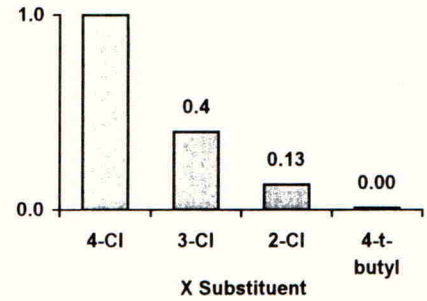


Figure 1. Ranking of substituents based on $^{1}/EC_{50}$ (*Trichoplusia ni*) for compound with substituent X divided by EC_{50} for compound with X=4-Cl.

It was further demonstrated that dichloromethyl was near the optimum activity for the group attached to the centre of the imidate linkage. However, photostability studies showed that the dichloroacetimidates rapidly degraded upon irradiation, suggesting robust effectiveness would not be achieved if the compounds were tested in the field. Photostability was significantly improved and the overall insecticidal efficacy was enhanced as well by replacing the dichloromethyl group with an isopropyl group. Alternative replacements for this central group resulted in a significant loss of activity (>4X) for even the smallest changes.

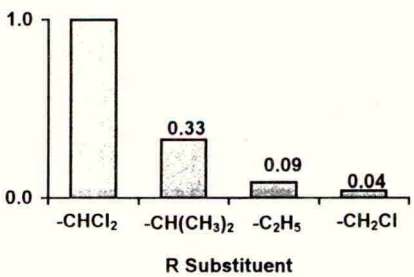


Figure 2. Ranking of substituents based on $^{1}/EC_{50}$ (*Trichoplusia ni*) for compound with substituent R divided by EC_{50} for compound where R=-$CHCl_2$.

ENVIRONMENTAL AND TOXICOLOGY PROFILE

Environmental persistence of these N-aryl imidates would not be a significant problem; the soil half life of a typical imidate of this chemical class is less than two days, with chemical hydrolysis of the carbon nitrogen double bond as the major mode of decomposition in all cases studied. Fish toxicity is also extremely low with no symptomology at all observed when Koi carp were exposed to a continuously replenished concentration of 10mg/l of Compound 3.

Compound 3

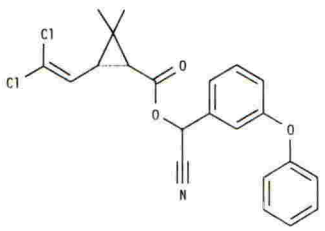
Acute mammalian toxicity of imidates is also very low with oral and dermal MLD's of over 1000mg/kg for Compound 3 when tested on either male or female rats.

STRUCTURE ACTIVITY STUDIES: OPTIMISATION OF THE PHENOXY BENZYL SUBSTITUENT

The phenoxy benzyl substituent of the imidate structure is familiar as a common component of pyrethroid ester insecticides such as permethrin. Attempts to prepare simpler analogues by removal of the phenoxy substituent virtually eliminated all insecticidal activity of these compounds, further supporting the original premise that these compounds are related to pyrethroids. Insect electrophysiology experiments have also shown that these compounds act on sodium channels in a manner analogous to pyrethroids. Various substituted benzyl alcohols were prepared without giving activity approaching the early lead (Compound 1).

Exploration of other moieties known to substitute effectively for a phenoxy benzyl substituent in various pyrethroid series gave mixed results. For example, analogues containing the alcohol substituent from bioresmethrin gave no or poor activity whilst reasonable levels of activity were obtained with compounds containing the corresponding substituents taken from bifenthrin or tefluthrin. However none of the compounds prepared during this phase of the work displayed advantages over analogues containing the phenoxy benzyl moiety.

An obvious structural ploy, incorporation of the α-cyanophenoxybenzyl substituent found in cypermethrin, was never tested for possible improvement of activity in these imidate insecticides. Despite numerous synthetic attempts by a variety of methods, appropriate analogues could not be successfully prepared, suggesting poor intrinsic chemical stability of such compounds.



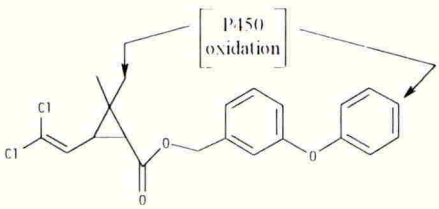
cypermethrin

Consideration was then given to the hypothesis that it might be possible to enhance activity of this series by blocking metabolic breakdown of the molecule directed at the phenoxy benzyl substituent.

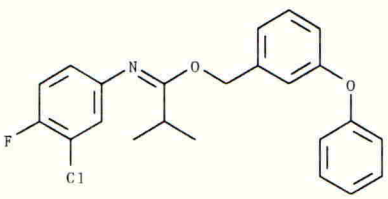
Metabolic blocking strategy

As a consequence of our work, replacement of the ester linkage of a pyrethroid with an imidate was believed to be a way to enhance metabolic stability of sodium channel effectors *in vivo* but other modes of metabolism are known to cause breakdown in pyrethroids. In particular, oxidation by cytochrome P450 systems would give rise to hydroxylated species which would undergo further conjugation and excretion, losing biological activity along the way. Conventional ester pyrethroids, e.g. permethrin, are known to undergo hydroxylation on

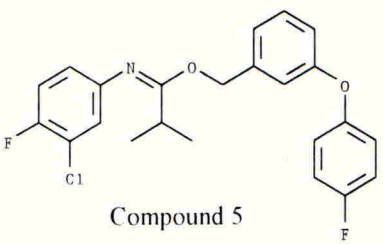
the germinal dimethyl substituents on the cyclopropane ring and most notably on the 4 prime position of the 3 phenoxy benzyl group.



This imidate series did not contain the equivalent of the cyclopropane germinal methyls, so blocking the most likely sites for hydroxylation in the phenoxy benzyl substituent with fluorine was explored as a strategy for improving the activity in the series. Substitution with fluorine at the 4' position was known in conventional pyrethroids but had not been observed to give improved activity. This strategy was employed with Compound 4 to give the additionally fluorinated, "metabolically blocked" Compound 5.



Compound 4



Compound 5

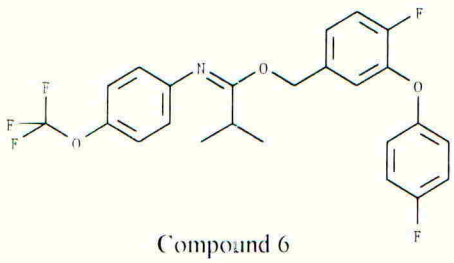
Biological evaluation of Compound 5 revealed a dramatic (10X) improvement in activity against *Trichoplusia ni*, the lepidopteran used for routine evaluation. Smaller, but significant, improvements were also observed against *Heliothis virescens* and other Lepidoptera species (Table 1). Curiously, the effect was only observed to work on Lepidoptera and not on Homoptera, *Musca spp.* or *Blattella spp.*

Table 1. Effectiveness of metabolic-blocking on susceptible Lepidoptera (EC_{50} concentrations of test solution in mg/l).

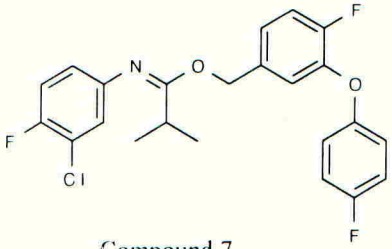
Compound	*Trichoplusia ni*	*Heliothis virescens*	*Spodoptera exigua*
4	3	7.5	18
5	0.3	5	9

Further "metabolic blocking" studies were conducted by further selective fluorination of Compound 5. All but one of the remaining hydrogen positions were replaced, one by one, with a second fluorine atom. This study led to the observation of no further enhancement of activity except when the fluorine is placed on the 4- position of the benzyl ring, a position known to improve activity with conventional pyrethroids. Compounds 6 & 7, prepared during these studies, were subsequently shown to be the most potent in this imidate series of insecticides for controlling Lepidoptera.

Data indicating the progression in activity provided by selective fluorination - Compound 4 →(5) →(7) - are provided in Table 2.



Compound 6



Compound 7

OPTIMISATION OF ACTIVITY AGAINST RICE PESTS

The low fish toxicity of these imidate insecticides suggested rice as a potential market opportunity. Several compounds resulting from previous studies directed largely at Lepidoptera were tested against a variety of important rice pests, including *Nephotettix cincticeps*, *Nilaparvata lugens* and *Chilo suppressalis*. Further synthesis was specifically directed at improving homopteran activity as well. Although data for individual compounds varied widely for different test species, Compound 7 represented nearly optimal activity when viewed across a broad spectrum of important pests (Table 2).

Table 2 Comparative relative potencies of imidate insecticides on three pest species (the ratio of the two doses necessary to elicit a 50% response level based on a logit parallel line analysis).

Compound	*Heliothis virescens*	*Chilo suppressalis*[c]	*Nephotettix cincticeps*[c]
4	0.007[a]	0.41	0.27
5	0.011[a]	0.56	0.27
7	0.028[a] (2.2[b])	1.6	0.75

[a] Potency relative to λ–cyhalothrin
[b] Potency relative to profenofos
[c] Potency relative to etofenprox

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MB-599, A NEW SYNBERGIST IN PEST CONTROL

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ABSTRACT

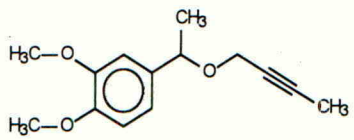
MB-599 (proposed common name: Verbutin), is a novel synergist which increases the activity of insecticides against a broad range of insect pests important in plant protection, public health and veterinary fields of use. It was selected from hundreds of newly synthesised compounds by the level and selectivity of synergistic action. Efficacy studies carried out on *Leptinotarsa decemlineata, Heliothis armigera, Aphis gossypii, Rhopalosiphum padi, Acyrthosiphon pisum, Tetranychus urticae, Musca domestica* and *Blattella germanica* have demonstrated the excellent synergistic activity of MB-599 on the potency of different insecticides (carbofuran, carbaryl, permethrin, tetramethrin, beta-cypermethrin, fipronil), aphicides (pirimicarb, triazamate, imidacloprid) and miticides (fenazaquin, tebufenpyrad) MB-599 used as foliar spray additive in field conditions multiplied the efficacy of active ingredients 2-4-fold even at the 1:1 insecticide:synergist ratio. This high synergist potency allows a decrease in the dose of treatments. MB-599 is classified as slightly toxic and has proved to be non-mutagenic by Ames and SCE tests.

INTRODUCTION

The potency of insecticides is limited by several metabolic detoxification mechanisms. To improve the insecticide activity different metabolic inhibitors can be used as synergists, depending on actual detoxification enzymes involved. In spite of the high potential benefits of these compounds their field application is limited by some drawbacks. The cost-efficacy ratio of insecticide:synergist and the spectrum of activity (both in terms of targeted insects and synergised insecticides) are the most critical elements from commercial point of view (Raffa & Priester, 1985). MB-599 is a new synergist which was discovered and patented by CHINOIN (Árvai *et al.*, 1995). The compound is a representative member of a new class of synergist containing an alkynyl side-chain of six atoms very likely inhibiting the metabolic detoxification via cytochrome P-450 monooxygenation. The mode of action has been investigated by 3D docking calculation (Keserû, unpublished results). The structure of the developed compounds was optimised by comparative structure-activity relationship studies (Bertók, unpublished results). Among the synthesised analogues several promising candidates were found which exhibited high synergist potency both on susceptible and resistant populations. Consequently MB-599 seems to be an effective tool for delaying and suppressing the resistance of insect populations (Pap *et al.*, 1996). In this paper the toxicological profile, biological activity and field performance of MB-599 are presented.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name:	1-(3,4-dimethoxyphenyl)-ethyl but-2-ynyl ether
Code number:	MB-599
Common name (proposed):	Verbutin
Molecular formula:	$C_{14}H_{18}O_3$
Molecular weight:	234.28
Structural formula:	



Appearance:	Colourless or slightly yellow oil
Stability:	Degradation was detected in acidic condition (pH<2)
Odour:	Faint chemical
State:	Viscous liquid
Boiling point	120 °C/0.1 Hgmm
Solubility:	Practically insoluble in water, less soluble in hexane, well soluble in most organic solvents
Heat stability:	Stable up to 100 °C, degradation was detected over 100 °C
Identity:	1H and C13NMR, IR, elementary analysis
Homogeneity:	TLC, GC, boiling point and refractive index
Purity:	GC: CP9000, CP-SIL-5CB, 60m x 0.53mm, N_2 5ml/min, FID
n_D^{25}:	1.528

TOXICOLOGY

Based on preliminary toxicity data, MB-599 has no acute hazard in normal use. (Slightly hazardous / Class III, according both to WHO and EPA toxicity classification.)

Acute oral LD_{50}:	mouse	male	3619 mg/kg
		female	2495 mg/kg
	rat	male	1290 mg/kg
		female	697 mg/kg
Acute dermal LD_{50}:	rat	male	> 2000 mg/kg
		female	> 2000 mg/kg
Eye irritation:	rabbit		Slight irritant
Skin irritation:	rabbit		Non irritant
Mutagenicity:	Ames test		Negative
	SCE in Chinese Hamster Ovary cells		Negative

BIOLOGICAL PROPERTIES

Laboratory evaluation

Synergist potency of MB-599 and commercial synergists, ENT-8184 as well as PBO, was compared on a susceptible housefly strain (*Musca domestica WHO/SRS*) by topical application with fixed dose (1000 ng/fly) synergist and at the 1:0.5, 1:1, 1:2 and 1:5 ratios of insecticide:synergist. In general, synergism increased with higher insecticide:synergist ratio and was consistently greater at the level of LD_{95} (SR_{95}) than LD_{50} (SR_{50}). MB-599 showed the highest synergism of the insecticides tested (tetramethrin, permethrin and carbofuran) in comparison to such well established synergists as PBO and ENT-8184. MB-599 elevated the insecticide potency of the insecticides significantly even at the 1:0.5, 1:1 and 1:2 ratios which are desirable from the cost-efficay point of view as is shown in Table 1.

Table 1. Synergism of tetramethrin, permethrin and carbofuran on susceptible housefly

Ratio of insecticide to synergist	Synergist					
	ENT-8184[a]		PBO[b]		MB-599	
	SR_{50}	SR_{95}	SR_{50}	SR_{95}	SR_{50}	SR_{95}
	Synergist Ratio[c]					
Tetramethrin[d]						
1:0.5	2.2	2.2	3.3	4.7	5.8	7.1
1:1	2.3	3.4	4.7	6.9	6.8	9.8
1:2	3.4	3.9	5.4	8.0	6.6	9.2
1:5	3.6	4.4	8.5	8.8	8.0	11.9
1000[g]	1.2	1.4	4.7	2.9	10.4	14.4
Permethrin[e]						
1:0.5	0.8	0.9	1.3	2.6	1.6	2.0
1:1	0.7	0.8	1.7	1.7	1.9	2.5
1:2	1.0	1.5	1.5	2.6	1.8	2.8
1:5	1.0	1.5	1.9	2.8	2.2	4.0
1000[g]	0.7	1.1	3.7	3.8	5.2	6.4
Carbofuran[f]						
1:0.5	1.0	1.5	1.1	1.9	2.8	6.2
1:1	0.9	1.7	1.5	3.2	3.9	5.2
1:2	1.3	2.2	1.8	4.3	6.1	7.8
1:5	1.6	3.1	2.8	6.6	12.2	17.7
1000[g]	2.0	2.0	8.7	12.3	34.8	87.9

a) ENT-8184: N-(2-ethylhexyl)-8,9,10-trinorborn-5-ene-2,3-dicarboxamide (MGK 264)
b) PBO: piperonyl butoxide
c) SR_{50}, SR_{95}: LD_{50} or LD_{95} of insecticide alone / LD_{50} or LD_{95} of synergized insecticide
d) LD_{50}: 2.3 µg/fly LD_{95}: 15.3 µg/fly
e) LD_{50}: 78.2 ng/fly LD_{95}: 379.0 ng/fly
f) LD_{50}: 173.8 ng/fly LD_{95}: 1195 ng/fly
g) 1000 ng/fly fixed dose of synergist was applied

Table 4. Synergist activity against two-spotted spider mite (*Tetranychus urticae*)

Treatment	Acaricide: synergist ratio	Exposure time (h)	LC_{50} (mg/l)	LC_{95} (mg/l)	SR_{50}	SR_{95}
Carbofuran	1:0	24	163.9	799.9	-	-
Carbofuran + MB-599	1:1	24	55.5	286.8	3.0	2.8
	1:2	24	33.5	93.7	4.9	8.5
	1:4	24	27.5	67.0	6.0	11.9
Fenazaquin	1:0	3	>1000	-	-	-
	1:0	24	41.9	801.3	-	-
Fenazaquin + PBO	1:1	3	326.7	>1000	>3.1	-
	1:1	24	20.4	371.8	2.1	2.2
Fenazaquin + MB-599	1:1	3	68.1	280.2	>15	-
	1:1	24	31.3	174.8	1.3	4.6
Tebufenpyrad	1:0	3	>1000	-	-	-
	1:0	24	63.4	>1000	-	-
Tebufenpyrad + PBO	1:1	3	115.9	1081	>9	-
	1:1	24	35.7	118.0	1.8	>8.5
Tebufenpyrad + MB-599	1:1	3	61.8	658.7	>16	-
	1:1	24	22.3	141.8	2.8	>7.0

Susceptible laboratory strain was used in the test
LC_{50} (mg/kg) was determined from 48 h mortality data
Excised bean leaf dipped in test solution for 5 s infested with female mites

Field performance

Field studies were conducted with Colorado potato beetle (*Leptinotarsa decemlineata*) and pea aphid (*Acyrthosiphon pisum*) to support the laboratory results and to confirm the field performance of MB-599. MB-599 was used in field trials in a 10 % EC formulation (proposed trade name: Censor), as a tank mix prepared before spraying. The effective dose of carbofuran against *L. decemlineata* could be reduced by 50 percent when applied together with MB-599 in a ratio of 1:1 (Table 5). Tests on *A. pisum* gave 2-4-fold improvement of pirimicarb, triazamate, imidacloprid, fipronil and carbofuran in terms of efficacy (Table 6).

Table 5. Control of *Leptinotarsa decemlineata* on potato at Környe, Hungary 1996

Treatment	Dose g a.i./ha	Average number of beetles/leaf At treatment	1-DAT	3-DAT	5-DAT
Carbofuran	320	18.1	1.1	0.2	0.1
	160	25.6	3.7	3.0	2.6
	100	22.6	7.8	5.1	4.5
Carbofuran + MB-599	160 + 160	23.0	1.1	0.8	0.7
	100 + 100	17.8	4.1	2.8	1.6

Treatment: 16 June; Spray volume: 300 l/ha, Plot size: 100 m^2; Replicate: 2

Table 3. Synergist activity of MB-599 on different pest species

Treatment	Life stage	LD_{50}	LD_{95}	SR_{50}	SR_{95}
German cockroach (*Blattella germanica*)[a] (Synergist was administered in 1 µg/insect)					
Carbofuran	adult	0.52	2.9	-	-
Carbofuran + PBO	adult	0.51	2.8	1.0	1.0
Carbofuran + ENT-8184	adult	0.39	1.4	1.3	2.1
Carbofuran + MB-599	adult	0.23	1.0	2.3	2.9
Carbofuran	third instar	0.93	4.2	-	-
Carbofuran + PBO	third instar	0.59	3.4	1.6	1.2
Carbofuran + ENT-8184	third instar	0.33	4.0	2.8	1.1
Carbofuran + MB-599	third instar	0.35	3.4	2.7	1.2
Beta-cypermethrin	adult	0.045	0.36	-	-
Beta-cypermethrin + PBO	adult	0.022	0.31	2.0	1.2
Beta-cypermethrin+ ENT-8184	adult	0.030	0.29	1.5	1.2
Beta-cypermethrin+ MB-599	adult	0.021	0.14	2.1	2.6
Beta-cypermethrin	third instar	0.021	0.30	-	-
Beta-cypermethrin+ PBO	third instar	0.015	0.24	1.4	1.3
Beta-cypermethrin+ ENT-8184	third instar	0.008	0.11	2.6	2.7
Beta-cypermethrin+ MB-599	third instar	0.006	0.09	3.5	3.3
Colorado potato beetle (*Leptinotarsa decemlineata*)[b] (Insecticide:synergist ratio = 1:2)					
Carbofuran	adult	5.1	50.3	-	-
Carbofuran + MB-599	adult	1.7	16.5	3.0	3.0
Carbofuran	fourth instar	0.8	5.1	-	-
Carbofuran + MB-599	fourth instar	0.5	1.9	1.6	2.7
Carbaryl	fourth instar	46.2	>500	-	-
Carbaryl + MB-599	fourth instar	11.8	43.5	3.9	>10
Cotton bollworm (*Heliothis armigera*)[c] (Synergist was administered in 1 µg/insect)					
Carbofuran	second instar	>5.0	-	-	-
Carbofuran + PBO	second instar	0.25	6.1	>20	-
Carbofuran + MB-599	second instar	0.03	0.35	>150	-
Cotton aphid (*Aphis gossypii*)[d] (Insecticide:synergist ratio = 1:1)					
Carbofuran	mixed	7.3	12.9	-	-
Carbofuran + MB-599	mixed	2.5	8,6	2.9	1.5
Pirimicarb	mixed	7.9	20.6	-	-
Pirimicarb + MB-599	mixed	3.1	11.2	2.5	1.8
Triazamate	mixed	23.0	42.8	-	-
Triazamate + MB-599	mixed	17.7	33.4	1.3	1.3
Oat aphid (*Rhopalosiphum padi*)[e] (Synergist was applied at 30 ppm concentration)					
Carbofuran	mixed	0.63	1.13	-	-
Carbofuran + MB-599	mixed	0.41	0.39	1.5	2.9
Imidacloprid	mixed	0.059	0.125	-	-
Imidacloprid + MB-599	mixed	0.029	0.060	2.0	2.1
Pirimicarb	mixed	2.18	17.1	-	-
Pirimicarb + MB-599	mixed	0.55	1.1	4.0	15.5

a) Susceptible laboratory strain, male, topical application, 48 h mortality, LD values are in µg/insect
b) Field collected population, topical application, 24 h mortality, LD values are in µg/insect
c) Field collected population, topical application, 24 h mortality, LD values are in µg/insect
d) Collected from greenhouse, tarsal contact test on sprayed cucumber leaf, 24 h mortality, LC values are in mg/l
e) Maintained in greenhouse, tarsal contact test on sprayed oat leaf, 24 h mortality, LC values are in mg/l

Synergistic activity was also investigated in house fly strains showing different susceptibility. Data in Table 2 demonstrate that the potency of MB-599 and PBO to permethrin was in a similar range in every housefly strain independent of their resistance status. Moreover MB-599 showed much higher level of synergism on carbofuran than PBO. The toxicity of carbofuran was increased 35-, 615-, 308-, and 2083-times in WHO/SRS, CHXSEL, MD-IX and CARBSEL strains, respectively.

The comparative studies with the known potent reference compounds proved that MB-599 is significantly better than the members of the alkynyl synergist family for example MB-603 which is claimed to be the most active analogue (Brown *et al.*, 1996) which is presented in Table 2.

Table 2. Synergism of MB-599, compared to reference synergists, on housefly strains with different susceptibilities. (Each synergists was applied at 1000 ng/fly fixed dose.)

Compound	Housefly strains							
	WHO/SRS[a]		CHXSEL[b]		MD-IX[c]		CARBSEL[d]	
	LD_{50}[e]	SR_{50}	LD_{50}	SR_{50}	LD_{50}	SR_{50}	LD_{50}	SR_{50}
Carbofuran	174	-	15375	-	25600	-	100000	-
Carbofuran + PBO[f]	20	9	-	-	450	57	7500	13
Carbofuran + MB-603[g]	7	25	-	-	-	-	-	-
Carbofuran + MB-599	5	35	25	615	83	308	48	2083
Permethrin	28	-	882	-	45672	-	3132	-
Permethrin + PBO	7	4	304	3	6371	7	245	13
Permethrin + MB-599	9	3	339	3	5951	8	239	13

a) Susceptible strain
b) Beta-cypermethrin selected strain (Pap & Tóth, 1995)
c) Field-collected multi-resistant strain (Pap & Farkas, 1994)
d) Carbofuran-selected strain (Pap *et al.*, 1996)
e) LD_{50} is expressed as ng/fly
f) Piperonyl butoxide
g) 1,2,4-trichloro-3-(2-propynyloxy)benzene

Laboratory investigation revealed that MB-599 exhibited superior synergistic activity with several insecticides against German cockroach (*Blattella germanica)*, Colorado potato beetle (*Leptinotarsa decemlineata)*, cotton bollworm (*Heliothis armigera)*, cotton aphid (*Aphis gossypii)* and oat aphid (*Rhopalosiphum padi)* (Table 3).

The potency of MB-599 on acaricides was tested against two-spotted spider mite (*Tetranychus urticae*) by contact bioassay. MB-599 improved not only final toxicity of specific acaricides, fenazaquin and tebufenpyrad, but greatly enhanced the knockdown effect (Table 4).

Table 6. Control of pea aphid (*Acyrthosiphon pisum*) on peas in Fejér county, Hungary (1996)

Treatment	Dose (g a.i./ha)	E (%)[a)]	Treatment	Dose (g a.i./ha)	E (%)
Pirimicarb	250	93.8	Triazamate	100	99.0
	125	89.4		50	96.8
	80	86.3		33	94.6
	60	81.5		25	92.3
Pirimicarb+MB-599	80+80	95.5	Triazamate+MB-599	33+33	97.7
Fipronil	240	94.6	Imidacloprid	350	100
	120	93.2		180	100
	80	84.2		120	98.4
	60	81.0		90	96.2
Fipronil+MB-599	120+120	95.7	Imidacloprid+MB-599	120+120	100.0
Carbofuran	320	100			
	160	99.1			
	110	98.0			
	80	94.0			
Carbofuran+MB-599	110+110	100.0			

Efficacy % is expressed by Henderson & Tilton (1955) at 2-DAT.
Average number of aphids/leaf was 94.7 and 109 on untreated control plot before and two days after treatment, respectively. Treatment 5 July; Spray volume: 300 l/ha; Plot size: 10 m^2

CONCLUSIONS

MB-599 is a new potent synergist acting on a broad spectrum of insecticides. Its advantages include dose-reduction of active ingredients in a cost-effective way and a real possibility to manage resistant insect populations. It has a good toxicological profile and has a low impact to environment.

ACKNOWLEDGEMENTS

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RH-2485: A NEW SELECTIVE INSECTICIDE FOR CATERPILLAR CONTROL

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ABSTRACT

RH-2485 is a second generation ecdysone agonist insecticide under development by Rohm & Haas. This compound controls a broad range of lepidopterous larvae at low use rates primarily via ingestion. RH-2485 also exhibits selective contact, ovicidal, and root-systemic activity. RH-2485 binds to the insect's ecdysone receptor within a few minutes of ingestion, arrests larval feeding and induces a premature lethal moult within a few hours, and kills the insect within a few days. Global laboratory and field trial evaluations of RH-2485 have shown excellent control of key caterpillar pests of vine, treefruits,vegetables, and row crops at application rates ranging from 20 to 300 g ai/ha. RH-2485 is selective toward pollinators, arthropod predators, and insect parasitoids. RH-2485 is non-phytotoxic. It is ideal for use in integrated pest management.

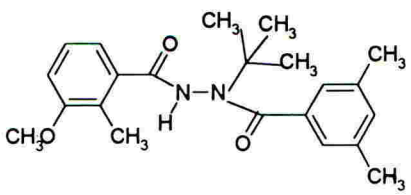
INTRODUCTION

Caterpillars are key pests on several crops. Extensive use of broad-spectrum insecticides to control these pests has affected the arthropod natural enemies leading to the resurgence of secondary pests, such as aphids, mites, and leafhoppers. Several of these broad-spectrum products are also toxic to the mammals as well as to other non-target organisms. Rohm & Haas, through its focused research, announced tebufenozide (RH-5992, Heller *et al.*, 1992), the first novel ecdysone agonist insecticide with excellent safety to non-target species and halofenozide (RH-0345, James *et al.*, 1995), a related product for control of scarabeid grubs in soil. RH-2485 is the third more potent member of this chemistry. This paper describes the chemical, physical, toxicological, and insecticidal properties of RH-2485 as well as its field performance and safety to non-target beneficial arthropods.

CHEMICAL AND PHYSICAL PROPERTIES

Code Number: RH-2485 RH-112,485

Structural formula:



Molecular formula:	$C_{22}\ H_{28}\ N_2\ O_3$
Molecular weight:	368
Chemical name (IUPAC):	N'-tert-butyl-N'-(3,5-dimethylbenzoyl)-3-methoxy-2-methylbenzohydrazide
ISO name:	Methoxyfenozide (proposed)
Physical state:	White powder
Melting point:	204 - 205 °C
Log P (shake flask):	3.7
Odour:	None
Vapour pressure:	$< 4.0 \times 10^{-8}$ torr at 25 °C
Stability:	Stable at 25 °C Stable to hydrolysis at pH 5, 7 and 9
Solubility:	Water: <1 mg/l DMSO: 11 % Cyclohexanone: 9.9% Acetone: 9%
Formulation:	2 F (2 lb/gal or 240 g/l, aqueous flowable) 80 WP (weight %, wettable powder)

MAMMALIAN TOXICOLOGY

Acute oral LD_{50} (rat, mouse):	>5000 mg/kg
Acute dermal LD_{50} (rat):	>2000 mg/kg
Eye irritation (rabbit):	non-irritating
Skin irritation (rabbit):	slight irritation
Dermal sensitization (guinea pig):	negative
Acute inhalation LC_{50} (rat):	>4.3 mg/l
Ames assay:	negative

ENVIRONMENTAL TOXICOLOGY

Avian:	Mallard Duck, LC_{50} (8-day dietary):	>5620 mg/kg diet
	Bobwhite Quail, LC_{50} (8-day dietary):	>5620 mg/kg diet
Aquatic:	Bluegill Sunfish, acute LC_{50} (96 hours):	>4.3 mg/l
	Daphnia Magna, acute EC_{50} (48 hours):	3.7 mg/l

Other studies:	Honey Bee (oral and contact), acute LD_{50}:	>100 µg/bee
	Earthworm LC_{50} (14 days):	>1213 mg/kg soil

MODE OF ACTION

RH-2485 is primarily taken up by insects via ingestion. The compound also shows selective uptake via contact, as well as some ovicidal activity. RH-2485 binds to the ecdysone (insect's natural moulting hormone) receptor protein of lepidopterous larvae, leading to feeding cessation and induction of a premature lethal moult. This mode of action is similar to that of tebufenozide and RH-0345 (halofenozide), but quite different from those of other insect growth regulators, such as benzoylphenylureas and juvenoids, where the insects continue to feed until their normal moulting cycle.

LABORATORY INSECTICIDAL PROPERTIES

The laboratory insecticidal properties were determined by exposing test insects to RH-2485 via feeding, contact, and root-systemic routes (Table 1).

Table 1. Laboratory insecticidal activity of RH-2485 against selected Lepidopterans

Test species	Test stage	Test method*	DAT,	LC50, mg ai/l
Spodoptera eridania	L-3	FS	3	0.13
Spodoptera eridania	L-3	SC	3	11.00
Spodoptera eridania	L-3	RS	7	0.85
Adoxophyes sp.	L-3	LD	6	0.25
Chilo suppressalis	L-2	LD	7	1.20
Cnaphalocrocis medinalis	L-3	LD	5	0.18
Heliothis virescens- Pyr-sus. **	L-1	LD	4	2.00
Heliothis virescens- Pyr- res. **	L-1	LD	4	1.60
Homona magnanima	L-2	LD	6	0.22
Spodoptera litura	L-2	LD	6	0.36
Cydia pomonella	L-1	DI	10	0.10
Helicoverpa zea	L-1	DI	5	0.10
Spodoptera exigua	L-1	DI	5	0.06

*DI= Diet incorporation test (LC50 in mg ai/kg diet); LD= Leaf dip; FS= Foliar spray; SC= Spray contact (LC50 in mg ai/l solution); SI= Soil incorporation; RS= Root systemic (LC 50 in mg ai/l of soil); DAT= Days after treatment; **LC50 for cypermethrin: Pyr-susceptible, 2.2 mg ai/l; Pyr-resistant, 80 mg ai/l

The feeding tests were conducted by either foliar spray (FS), leaf dip (LD), or by the diet incorporation (DI) method. The contact test for foliar pests was done by spray contact (SC), where the insects were directly sprayed with test solutions, air-dried, and allowed to feed on untreated foliage. The contact test for the soil pests was done by soil incorporation (SI). The root systemic (RS) test was conducted by drenching the potted host plants with test solutions, and bioassaying the foliage one week after the soil drench.

The test results indicate that RH-2485 is highly active against larval lepidoptera by oral uptake and to a lesser extent by contact. RH-2485 is root-systemic, but does not appear to have translaminar or phloem systemic properties. RH-2485 is equally effective against pyrethroid-susceptible as well as pyrethroid-resistant (35X resistance to cypermethrin) *H. virescens*. The compound showed no activity against larval and/or adult non-lepidopterans, such as, *Empoasca fabae*, *Nephotettix cincticeps*, *Epilachna varivestis*, *Myzus persicae*, *Tetranychus urticae* (foliar test at 600 mg ai/l), *Diabrotica undecimpunctata howardi* and *Meloidogyne incognita* (Soil incorporation test at 8 mg ai/l in soil). Greenhouse and field trials indicate that RH-2485 is not phytotoxic.

FIELD EVALUATIONS

The following field trials illustrate the performance of RH-2485 versus representative lepidopteran species:

Apple

Cydia pomonella (codling moth) is a major pest in many apple-growing countries. *Argyrotaenia ljungiana* (=*A. pulchellana*, tortrix moth), is an apple and vine pest in Europe but the related *A. velutiana* (red-banded leafroller) and *A. citrana* (orange tortrix) attack the US crops. *Phyllonorycter blancardella* (spotted tentiform leafminer) is an apple pest in Canada, Europe and the US. In a season-long Italian apple field trial, RH-2485 at 6.0 - 9.6 g ai/ 100 l (108 - 173 g ai/ ha), provided a better control of *C. pomonella, A. ljungiana*, and *P. blancardella* than the reference treatment (Table 2).

Table 2. Control of *Argyrotaenia pulchellana, Cydia pomonella* and *Phyllonorycter blancardella* on apple, Canton (Verona), Italy 1995*

Product	Dose	% Damaged fruits, 30 DAT 6		Number of mines/leaf, 30 DAT 6
	(g ai/100 l)	*A. pulchellana*	*C. pomonella*	*P. blancardella*
RH-2485 2F	6	7.3	1.3	0.65
RH-2485 2F	9.6	4.5	1.3	0.69
quinalphos/ chlorpyriphos-m	37.5/ 40	13.8	3.3	1.23
Untreated		64.8	18.8	1.48

*Application: May 15th & 25th; July 5th & 17th; August 24th & September 4th; Spray volume: 1800 l/ha.

Peach

Cydia (=Grapholita) molesta (oriental fruit moth) tunnels into the shoots and fruits of many Rosaceae as well as a few Myrtaceae in Asia, Australia, Europe, the Middle East, New Zealand, and North & South America. Field results from Italy shows that RH-2485 at 9.6 g ai/hl, (192 g ai/ha) is more efficaceous than the reference treatment in reducing the fruit damage by the pest.(Table 3)

Table 3. Control of *Cydia molesta* on peach, Canton (Verona), Italy, 1995*

Product	Dose (g ai/100 l)	% damaged fruits at harvest (*C. molesta*, 7 DAT 4)
RH-2485 2F	9.6	2.2
azinphos-m/ carbaryl	50/ 90	9.2
Untreated		17.5

*Applications: June 13th; July 18th; August 1st & 17th. for RH-2485; June 13th, July 18th & August 1st for azinphos-m and August 17th for carbaryl. Spray volume : 2000 l/ha.

Grape

Clysia ambiguella (grape berry moth) is an European pest that destroys the flower clusters and green berries of grape. In a field trial conducted in France, RH-2485 at 60- 96 g ai/ha was more efficacious than the reference treatment (Table 4).

Table 4. Control of *Clysia ambiguella* on grape, Reuil (Champagne), France, 1994*:

Product	Dose (g ai/ha)	Number of *C. ambiguella* larvae/ bunch, 23 DAT 2
RH-2485 2F	60	0.20
RH-2485 2F	96	0.11
fenvalerate	75	0.26
Untreated		0.60

*Application: July 25th and August 7th. Spray volume: 250 l/ha.

Corn

Ostrinia nubilalis (European Corn Borer) is a major corn pest in Europe, North America and part of Asia and attacks other crops, including potatoes, beans, beets, celery, and pepper. In a trial conducted in the US (Seymour et al., 1996), field corn was artificially infested with neonate *O. nubilalis* prior to insecticide treatments at blister stage (R6). Evaluation at 40 DAT shows that RH-2485 is more efficacious than the reference treatments (Table 5).

Table 5. Control of *Ostrinia nubilalis* on corn, North Platte (Nebraska), USA, 1995*

Product	Dose (g ai/ha)	No. of cavities (40 DAT)	Total length of cavities in cm (40 DAT)	% of lodged stalks (48 DAT)
RH-2485 2F	140	0.55	0.55	1.25
chlorpyrifos	1120	1.20	2.55	14.38
methyl parathion	560	2.05	4.60	14.38
esfenvalerate	46	1.75	0.80	21.88
Untreated		3.55	8.30	46.25

*Application: August 9th. Spray volume: 200 l/ha.

SAFETY TO BENEFICIAL ARTHROPODS

RH-2485 has little or no toxicity toward beneficial insects. Against worker honeybee *Apis mellifera*, RH-2485 was non-toxic at 100 microgram/bee by topical application (this dose translates to 100 kg ai/ha). When *Colpoclypeus florus* adults (apple leafroller parasitoid) were subjected to simulated field application rate using a Potter Spray Tower, RH-2485 showed no toxicity to the parasitoid (Brunner & Doerr, 1995). Recent trials conducted in Germany by Bayer AG indicate that RH-2485, at a concentration of 96 mg/l, is selective to (no mortality and/or moulting abnormality) *Coccinella septempunctata* (adults, larvae & pupae; 25 trials), *Tytthaspis sedecimpunctata* (adults; 6 trials), *Cantharis fusca & Rhagonycha fulva* (adults; 6 trials) *Aphidius sp* (hatching out of aphid mummies; 4 trials), and *Forficula sp.(adults; 6 trials)*.

CONCLUSIONS

RH-2485 is a highly effective insecticide for selective control of most lepidopterous larvae at 20 to 300 g ai/ha. RH-2485 binds to the ecdysone receptor protein, resulting in feeding cessation and a premature lethal moult. RH-2485 demonstrates a low toxicity profile to mammals, birds, fish and non-target arthropods, such as insect pollinators, predators, and parasitoids. RH-2485 is an ideal selective insecticide for use in integrated pest management.

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D2341- A NOVEL AGENT TO CONTROL SPIDER MITES

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ABSTRACT

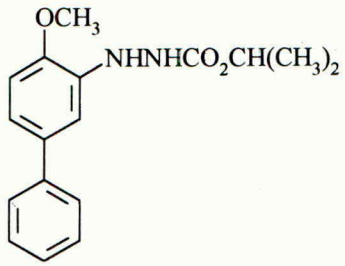
D2341 (N'-(4-Methoxy-biphenyl-3-yl)-hydrazinecarboxylic acid isopropyl ester) is a novel hydrazinecarboxylate acaricide discovered by Uniroyal Chemical for mite control in a range of agricultural and ornamental crops. The compound has a good toxicological and environmental profile. D2341 shows no cross-resistance with currently available acaricides and provides excellent control at low rates against all stages of tetranychid mites and motile forms of *Panonychus* species. Proposed field rates are 0.15-0.60 kg a.i./ha for the control of *Tetranychus urticae*. D2341 has outstanding knockdown and residual activity for control of many phytophagous mites. It shows minimal impact on beneficial insects and mites. It is therefore recommended in integrated pest management (IPM) programs. In trials on apples and citrus, D2341 has shown no crop injury at rates well in excess of proposed field rates.

INTRODUCTION

While conducting research into hydrazine derivatives, a new class of acaricidal compounds was discovered by Uniroyal Chemical. D2341 (N'-(4-Methoxy-biphenyl-3-yl)-hydrazinecarboxylic acid isopropyl ester) was selected as the most effective compound among many hydrazine derivatives (Dekeyser & McDonald, 1994, 1995; Dekeyser *et al.* 1994, 1995). D2341 is currently under development as a promising acaricide for citrus, apples, pears and cotton. In this paper, we describe the technical properties of D2341 and its miticidal activities under greenhouse and field conditions.

CHEMICAL AND PHYSICAL PROPERTIES

Code number	: D2341
Chemical name (IUPAC)	: N'-(4-Methoxy-biphenyl-3-yl)hydrazinecarboxylic acid isopropyl ester
(CA)	: Hydrazinecarboxylic acid, (4-methoxy-[1,1'-biphenyl]-3-yl)-, 1-methylethyl ester
Structural formula	:



Molecular formula	: $C_{17}H_{20}N_2O_3$
Melting point	: 120 - 121 °C
Molecular weight	: 300.36
Appearance at 20°C	: white crystals
Solubility	: 3.76 mg/l water at 20°C
Partition coefficient	: log P=3.4 at 25°C (n-octanol/water at pH 7)
Hydrolytic half-life	: 20 hours at pH 7 at 25°C
Photolytic half-life	: 17 hours at pH 5 at 25°C

TOXICOLOGY

Acute toxicity of the technical active ingredient:

Acute oral LD50 rat	: 5000 mg/kg body weight
Acute dermal LD50 rat	: >2000 mg/kg body weight
Skin irritation rabbit	: non-irritating
Eye irritation rabbit	: non-irritating
Ames test	: negative
Teratogenicity	: not teratogenic according to the data currently available
Mutagenicity	: mouse lymphoma negative

FORMULATIONS

Three formulations have been used: 50 WP (wettable powder), 20 SC (soluble concentrate) and 80 WDG (water dispersible granule).

BIOLOGICAL ACTIVITY

Acaricidal spectrum

D2341 shows high activity on phytophagous mites, such as *Tetranychus*, *Eutetranychus*, *Oligonychus* and *Panonychus* species, whereas it is harmless to predaceous mites, such as the phytoseiids *Amblyseius fallacis*, *Galendromus occidentalis* and *Zetzellia mali*.

Activity under greenhouse conditions

When applied to pre-infested leaves, technical D2341 sprayed to run-off on cowpeas showed activity against all life stages of *Tetranychus urticae* (Table 1).

Table 1. Comparative toxicity of D2341 and the commercial acaricides, propargite and clofentezine, as contact treatments to motile forms (larvae, nymphs and adults) and eggs of *Tetranychus urticae* on cowpeas.

Life Stage	LC50 (mg a.i./l) at 5 days		
	D2341	propargite	clofentezine
Adults	0.3	28	inactive
Nymphs*	0.3	14	inactive
Larvae	0.3	10	inactive
Eggs	12	inactive	4

*second, third and fourth stages.

Effect of temperature on activity

The acaricidal activity of D2341 remained constant over a wide temperature range. The LC50 values for D2341 were determined by infesting pre-treated cowpea plants with *Tetranychus urticae* and holding the plants at 15°C, 25°C and 35°C. D2341 showed no change in activity as the temperature was lowered (Table 2) compared with a significant decrease in activity reported for fenbutatin oxide and amitraz (Kyomura *et al.*, 1990). The relative insensitivity of D2341 to changes in temperature allows it to be used under a wide range of conditions.

Table 2. Effect of temperature on the activity of D2341 against adult *Tetranychus urticae* on cowpeas as a residual treatment.

Treatment	LC50 (mg a.i./l) at 6 days for 3 temperatures		
	35°C	25°C	15°C
D2341	15	11	25

FIELD PERFORMANCE

The biological activity of D2341 against spider mites in economically important apple and citrus crops was evaluated under field conditions in 1992-1994.

Apple

In 1992, a 50WP formulation of D2341 compared favourably to a 50SC formulation of clofentezine (Apollo) for the control of the European red mite, *Panonychus ulmi*, on apple (Figure 1). The rapid knockdown activity of D2341 is apparent in this graph.

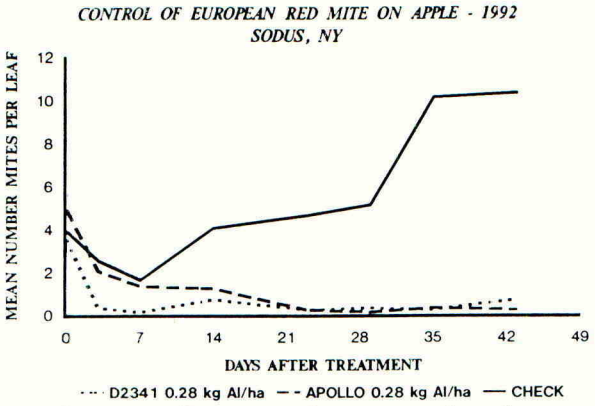


Figure 1. Field performance of D2341 against *Panonychus ulmi* on apples.

Citrus

In 1992, a 50WP formulation of D2341 compared favourably to a 25WP formulation of fenbutatin oxide (Vendex) for the control of the citrus red mite, *Panonychus citri*, on citrus (Figure 2).

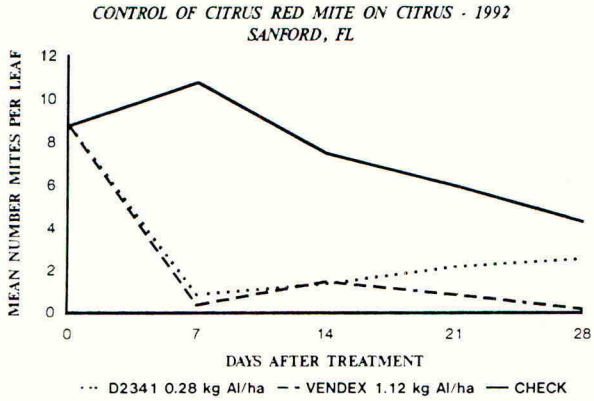


Figure 2. Field performance of D2341 relative to Vendex against *Panonychus citri* on citrus.

Effect on beneficials

D2341 demonstrated little toxicity to beneficial insects and predatory mites (Figure 3).

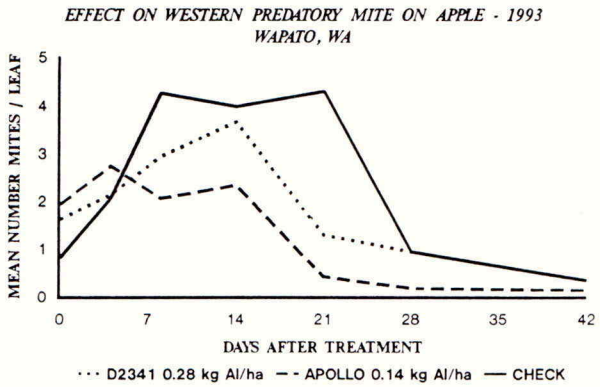


Figure 3. Effect of D2341 on the western predatory mite on apples.

Activity against resistant strains

Many established products that are currently used to control spider mites encounter resistance problems in several countries (Voss, 1988). D2341 is not cross-resistant with a range of conventional acaricides, such as pyridaben (Sanmite, Figure 4), fenpyroximate and tebufenpyrad. Its potency against mites resistant to existing acaricides offers a powerful tool for pest management in a variety of crops.

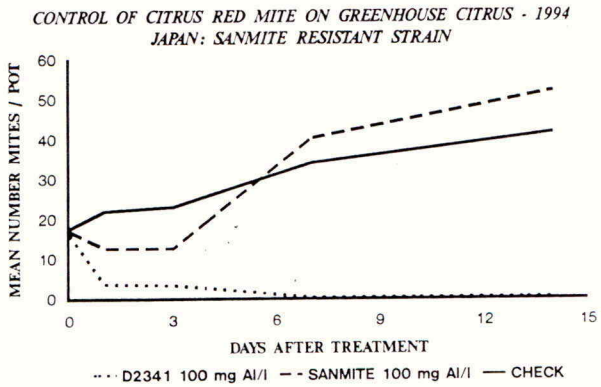


Figure 4. Performance of D2341 against a Sanmite resistant strain of citrus red mites.

Crop safety

D2341 has been evaluated at rates up to and including 1 kg a.i./ha in trials in citrus, apples and cotton in the U.S.A. and Japan. No injury has been reported on any crop tested.

CONCLUSIONS

D2341 is a highly selective new acaricide which has displayed good efficacy in field trial against economically important mite pests. The hydrazinecarboxylate compound is a member of a new chemical class that shows rapid knockdown, is not temperature sensitive, and controls mites resistant to other types of acaricides while sparing predator mites. This is particularly important in view of the widespread reduced sensitivity to existing acaricides (Wege & Leonard, 1994) and reports (Malezieux *et al.*, 1992) of population increases due to elimination of natural enemies.

ACKNOWLEDGEMENT

The authors would like to thank their many colleagues in Uniroyal Chemical who have participated in the D2341 project.

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SESSION 5B

FUSARIUM DISEASES – IMPORTANCE AND CONTROL OF A DIVERSE GROUP

Chairman	Dr D W Parry *Harper Adams Agricultural College, Newport*
Session Organiser	Dr G L Bateman *IACR-Rothamsted, Harpenden*
Papers	5B-1 to 5B-4

VASCULAR WILT DISEASES OF TROPICAL CROPS CAUSED BY *FUSARIUM OXYSPORUM*

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ABSTRACT

Fungal wilt diseases of plants caused by *Fusarium oxysporum* are of major economic importance in a wide range of tropical crops, such as banana, cotton, tomato, date palm and oil palm. Special forms (*formae speciales*) and races of the pathogen have been identified which, in some case, exhibit specificity to particular plant hosts. Special forms include *F. oxysporum* f.sp. *cubense* and *F. oxysporum* f.sp. *elaeidis*, the causal agents of vascular wilt of banana (Panama disease) and oil palm respectively, which are major constraints to production of the crops world-wide. Strains of *F. oxysporum* are also capable of surviving as saprobes. Control of vascular wilts in the tropics can be achieved using cultural practices but they are rarely very effective and their use by small scale farmers may be limited by practical and economic considerations. Chemical treatment is restricted by availability and/or cost of appropriate fungicides, particularly in developing countries, and is usually limited to high value cash crops (cut flowers). The use of plant resistance can be highly effective, and is frequently the primary means of disease control, given the soil-borne nature of these pathogens, the perennial nature of the crops and the plantation scale of operations. However, in many cases (including production of resistant material) the level of control is reduced by a lack of information on existing pathogenic variability. Rapid laboratory techniques for providing such information have been developed over a number of years at the International Mycological Institute, and the technology is now being transferred to East African counterparts for use in current and future local research programmes, especially on bananas.

MAJOR FUNGAL DISEASES OF TROPICAL CROPS CAUSED BY *FUSARIUM* SPP.

A number of fungal genera, including *Rhizoctonia, Phytophthora, Colletotrichum* and *Fusarium*, are capable of infecting a wide range of plants of agricultural importance in the tropics, resulting in significant crop losses annually. The genus *Fusarium* comprises a large number of species, many of which have pathogenic forms causing a range of diseases of both monocotyledonous and dicotyledonous tropical plant hosts. These include *F. moniliforme* (cotton boll rot, banana black heart, cereal stalk and ear rots), *F. graminareum* (blights, rots, scabs), *F. solani* (root and collar rots, cankers) and *F. pallidoroseum* (storage rots). Fusarium wilt of coffee, caused by *F. xylarioides*, is also a major cause for concern in parts of central Africa. Vascular wilts caused by *F. oxysporum* (also referred to as fusarium wilts) are some of the most widespread and destructive diseases of tropical crops. Following infection, the fungus colonises the vascular system, causing discoloration, leaf yellowing,

plant wilting and eventual death. Panama disease of banana, caused by *F. oxysporum* f.sp. *cubense*, caused major losses to the banana export trade in the 1950s, particularly in Central America, and today remains a major threat to production. In Uganda, an estimated 36% of the agricultural land is utilised for production of approx. 8.5 million tonnes of bananas per year, either as a monoculture or mixed with other crops, primarily for local consumption. There the disease has been recognised as one of the primary constraints to production. Annual oil palm losses of 10% per annum, attributed to *F. oxysporum* f.sp. *elaeidis*, have been reported in W. Africa (Renard *et al.*, 1972). In many instances the production of crops susceptible to fusarium wilt, such as the planting of oil palm in S.E. Asia (where the disease has not yet been reported), highlights the increasing need for the development and implementation of effective and sustainable disease management strategies. Precautions should also be taken to prevent the introduction of pathogens to disease-free areas.

PATHOGENIC VARIABILITY WITHIN *F. OXYSPORUM*

F. oxysporum is an extremely diverse species. Strains of the fungus are capable of surviving in soil (usually on plant debris) as saprobes, and can be pathogens of animals, including insects and man as well as plants. To date, the fungus has been found to infect over 150 plant species, which include not only many of the world's most important export/cash crops (e.g. oil palm, date palm, cotton, tomato, banana, chickpea) but also many of those, such as banana and oil palm, produced in developing countries as staple sources of food. Although collectively, species of *F. oxysporum* are capable of infecting such a wide range of plant hosts, individual strains have, in general, been assumed to be capable of infecting only the symptomatic host from which they have been isolated and have been delineated into special (pathogenic) forms, or *formae speciales*. However, subsequent research involving pathogenicity testing (although it has obvious shortcomings in relation to the processes of natural infection) has shown that many of these strains are in fact capable of infecting a number of, in some cases quite distinct, plant hosts. This has resulted in increasing confusion and doubt surrounding special form designations. Within individual special forms, a number of specialised pathogenic races may also have been identified. However, the methodology employed in assigning races is not consistent between individual special forms. While in some cases (e.g. *F. oxysporum* f.sp. *lycopersici*) race designations are based on the presence or absence of complementary host virulence and plant resistance genes, in others designations are based on the ability of a particular strain to infect either a particular plant cultivar, species or even genus (or some combination of these, e.g. *F. oxysporum* f.sp. *vasinfectum*). Furthermore, the number of races within a particular special form have risen as the number of potential hosts against which individual strains have been tested (and on which differences in strain pathogenicity and/or virulence have been observed) has increased. The result is that the term 'race' has lost credibility amongst many plant pathologists. Booth (1971) accepted 76 special forms within the species but Armstrong & Armstrong (1981) described more than 120 special forms and races. Although the variability within *F. oxysporum* has been recognised, particularly by plant pathologists, there are major practical limitations on our ability to determine the precise host range of strains, not only within designated special forms but also from other hosts. The concept of special forms (and pathovars) and races has been reviewed recently by Hawksworth (1994), who indicated that special form designations are inadequate, suffer from conceptual difficulties and are dangerous with respect to plant quarantine and biocontrol. Special form designations imply that more is known about the ability of a fungus

to attack a particular host than is actually based on scientific evidence. Brayford (1989), considered the *in vitro* identification of pathogenic strains of *F. oxysporum* to be one of the most important areas for research on the fungus, and recognised that a study of variation within the species as a whole was essential.

A number of factors may influence the apparent variability that exists within *F. oxysporum*. Inherent genetic variability does obviously exist within the pathogen itself, and a strong correlation with pathogenicity has been found in several cases, as in the case of *F. oxysporum* f.sp. *lycopersici* outlined above where a gene for gene relationship between pathogen and plant has been identified. Vegetative compatibilty grouping (VCG) has commonly been used to identify genetically distinct populations of *F.oxysporum,* and VCGs often correlate with field behaviour. Katan & Katan (1988) found that all isolates of *F. oxysporum* f.sp. *vasinfectum* Race 3 from two locations in Israel belonged to a single VCG and were not compatible with non-pathogenic strains from the rhizosphere of cotton. Puhalla (1985) identified 16 VCGs in the strains he studied and several corresponded with special forms, but with *F. oxysporum* f.sp. *cubense* where 15 VCGs have now been identified, several VCGs may be found within a race or a number of races within individual VCGs. However, it is important to note that the term race is not used in the strict sense for *F. oxysporum* f.sp. *cubense*, since the genetic basis for susceptibilty and resistance has not been determined. Modern methods of DNA analysis, including restriction fragment length polymorphisms and RAPD-PCR amplification, have also revealed correlations between genetic structure and pathogenicity and these provide useful tools for identifying genetically distinct groupings and clarifying the special form/race dilemma. Recent research undertaken at the International Mycological Institute (IMI) based on a number of number of approaches (molecular and biochemical) has indicated that differences observed between pathogenic races may be greater than those between special forms (Rutherford *et al.*, 1994).

Although development of disease will depend primarily on the nature of the pathogen and host concerned, it may also be influenced to varying degrees by environmental and other biotic factors. Pathogenic variation within recognised races may be particularly apparent. The banana cultivars IC2 and Bodles Altafort, while resistant to *F. oxysporum* f.sp. *cubense* Races 1 and 2 respectively in some regions of the world, are susceptible in others (Stover & Buddenhagen, 1986). Although these effects may be due to unrecognised inherent genetic variation within the pathogen, factors such as climate (temperature, light, humidity), soil type and the presence of other pests may predispose an otherwise resistant host to infection. *F. oxysporum* f.sp. *cubense* Race 4, the only race known to be capable of infecting Cavendish banana cultivars (on which much of the banana export trade relies), is presently confined to subtropical regions of the world. Cavendish cultivars have succumbed to the disease in tropical regions but it is believed that waterlogging, poor physical or chemical conditions may have been major contributing factors (Ploetz, 1990). Numerous macro- and microelements, including Ca, C and N, have been found to have a significant effect on the development of fusarium wilt on a wide range of plant hosts, as reviewed by Engelhard *et al.* (1989). Other pests may also influence development of the disease. Increased wilt incidence in the presence of root-knot nematodes has been observed in a number of crops, including cotton, tomato, chickpea and pigeonpea. The situation is complex, and results can differ greatly depending on nematode species, inoculum load, host genotype, soil type etc. Although physical damage may be responsible, in most cases the precise mechanisms of increased susceptibility have not been established. A pigeonpea variety, developed by ICRISAT in India and resistant to

fusarium wilt, remained resistant at most sites when released to growers in Malawi in 1987. However, resistance to the disease broke down at sites where the crop was subject to attack by root-knot nematode, and subsequent pot tests confirmed that the presence of the nematode did cause an increase in susceptibility (Hillocks & Marley, 1995).

IMPORTANCE OF PATHOGENIC VARIABILITY WITHIN *F. OXYSPORUM* TO DISEASE CONTROL

Pathogenic variability, particularly when it is as extensive as that found in *F. oxysporum*, is of major significance when considering possibilities for disease control. Knowledge of the inherent variability that may exist in a pathogen population, even within localised areas, is essential for both the development and implementation of biologically rational disease management strategies. In order to identify the most appropriate approaches to disease, a clear understanding of the form (including possible variants) and function of the pathogen in question is vital. This is particularly true where non-chemical approaches, such as the use of plant resistance, cultural and biological control, are to be considered. The use of chemical pesticides has, and will continue to be, an extremely effective means of disease control in many situations. However, several major drawbacks have been recognised, including a lack of specificity (resulting in damage to non-target organisms, possibly beneficial organisms), crop damage, accumulation of pesticide residues and development of pest tolerance/resistance. Although pesticides have been used for the control of *F. oxysporum* in developing countries in both temperate and tropical regions, their use is not widespread and is usually limited to soil fumigation and seed treatment (e.g. oil palm, Flood *et al.*, 1994) or for high value cash crops grown under controlled conditions (e.g. glasshouse carnation production). As public pressure continues to mount for a shift from pesticide usage towards more natural and environmentally friendly methods of pest control, the significance of studies on pathogenic variability are becoming more apparent.

The use of naturally occurring enemies (predators, parasites and pathogens) against major pests has obvious advantages over the use of pesticides but in comparison, the technology is still in its infancy. To date, the majority of research, and as a consequence most success, has been achieved against insect pests and weeds but the use of (microbial) biological control agents (BCAs) against plant pathogens has been increasing. Strategies employed are greatly affected, and often limited, by the characteristics of the crop to be protected (e.g. annual or perennial, seed or vegetatively propagated), farming systems employed and agronomic factors. In many cases the inability of the BCA to survive and/or multiply to sufficiently high levels is responsible for failure, particularly where prolonged periods of control are required as with perennial crops. The problem is particularly significant where control of *F. oxysporum* is required since the pathogen will continue to survive (in soil or on alternative hosts) and may re-infect the crop once BCA populations have declined. One notable exception to the use of biological control of *Fusarium* spp. is suppressive soil, whose importance to the successful production of many crops has long been recognised (Louvet *et al.*, 1981). Although the suppressive effect of soils on the development of fusarium wilt of banana in a number of regions has usually been associated with chemical and physical factors (Stover, 1990), in general the antifungal effect and resulting decrease in fusarium wilts is fundamentally microbiological in nature (Alabouvette *et al.*, 1993). Of those organisms isolated from such soils and investigated as potential biocontrol agents for fusarium wilts, fluorescent

pseudomonads and non-pathogenic *F. oxysporum* have consistently provided a satisfactory level of control, particularly with crops grown under controlled glasshouse conditions. Despite the recent advances, large scale commercialisation of non-pathogenic strains of *F. oxysporum* for control of fusarium wilts is inhibited by a number of problems, primarily the need to demonstrate that potential BCAs are not, and under natural conditions will not become, pathogenic to either the crop to be protected or other beneficial plants/ crops in the vicinity. Extensive field screening may be necessary, for example where control is required in mixed cropping systems (commonly employed in developing countries of the tropics), but is rarely feasible. While suppression may feasibly be due to the presence of other microorganisms, in particular non-pathogenic *F. oxysporum*, this aspect cannot be investigated fully until appropriate diagnostic tools become available for determining the extent of pathogenic variability present, for differentiating pathogenic and non-pathogenic forms and for rapidly and easily monitoring the changes in populations of both introduced and naturally occurring strains.

Cultural practices which may be employed for disease control fall into three main areas: (i) prevention of introduction of inoculum into the field, (ii) reducing inoculum survival/build-up and disease attack in or on soil and (iii) enhancing host resistance. Prevention of introduction of inoculum to disease-free areas is vital but is dependent on our knowledge of pathogenic forms present, the potential threat of alien forms to those crops grown and on our ability to readily detect particular pathogenic forms in planting material prior to its introduction to disease-free areas. Strict legislation is in place for *F. oxysporum* f.sp. *albedinis*, the causal agent of wilt of date, to prevent its introduction to countries where susceptible cultivars are grown. Although the pathogen may be rapidly detected and identified by cultural characteristics and vegetative compatibility grouping (following isolation from symptomless palm material and soil) other plants cultivated in date plantations in the region are known to act as symptomless carriers of the disease. On-farm practices and regional organisation may prevent introduction of inoculum into certain areas, limit the movement of diseased material and reduce inoculum and disease attack (through removal and destruction of inoculum sources, including volunteer plants, alteration of soil conditions, and employment of specific cropping practices to protect crops of major economic importance and eliminate alternative and reservoir hosts). These approaches provide a suitable alternative to disinfection with pesticides, and would benefit greatly from (and in some cases may be dependent on) knowledge of pathogenic forms present and the threat they present to particular crops. It is often assumed that cultural practices, such as organic amendments (and indeed the application of fungicides), have a major impact on *F. oxysporum* in soil and hence on wilt incidence. However, accurate assessment of their effects on pathogenic and saprobic populations, and in particular pathogenic forms and races, will not be possible until reliable and accurate techniques for investigating variability are available.

The use of plant resistance is considered to be the most important approach for future plant disease control in the tropics. Tropical regions often have an immensely rich plant flora, providing enormous scope for searching for, and utilising, naturally occurring resistance genes for breeding purposes. In countries such as Kenya, Uganda and Tanzania, literally hundreds of genetically distinct banana 'types' are grown. However, conventional methods of breeding for resistance are not suitable for all crops including bananas, and the process can be extremely complex and time consuming. Breeding for resistance may be based on a gene-for-gene interaction, where single, dominant (major) genes may be used to confer resistance to

particular pathogens possessing corresponding virulence genes. This approach may be highly effective, and breeding for resistance to specialised pathogens, including *F. oxysporum,* is often based on major genes. However, the selection pressure exerted usually leads to the development of new forms of the pathogen resistance, resulting in non-durable 'race'-specific resistance (Parlevliet, 1995). Alternatively, multiple gene (multigenic) resistance, based on a number of minor genes, is often effective against a wide range of pathotypes and, although more durable than major gene resistance, usually provides a lower level of control. Prolonged exposure to genetically heterogenous pathogen populations may result in multigenic resistance being expressed. However, resistance tends to segregate under field conditions, leading to variation in the levels of resistance and localised outbreaks of disease which may be severe and economically damaging, especially in areas of intensive cultivation. Multigenic resistance is also more difficult to manipulate in breeding programmes. To be effective over the long term, resistance breeding must be an ongoing process based on the continual incorporation of resistance genes for new races. The availability of rapid techniques for detecting and monitoring the distribution of particular forms of a pathogen such as *F. oxysporum* would greatly assist in the development and/or deployment of either monogenic or multigenic resistant plant material. This is true irrespective of whether particular pathotypes are causing significant damage at any particular time. As suggested by Crill (1977), the process of continually incorporating new race-specific resistance genes (i.e. gene stacking) as new races are identified (as in the case with *F. oxysporum* f.sp. *lycopersici)* may be counterproductive. Alternatively, genes conferring resistance to pathotypes which are no longer a serious threat should be removed to reduce selection pressure. As a result, pathogens such as *F. oxysporum*, which may persist for long periods in soil, may eventually be eliminated.

DEVELOPMENT OF *IN VITRO* TECHNIQUES FOR DIAGNOSING *F.OXYSPORUM*

On a broad scale, it is difficult or even impossible to develop and introduce effective legislation, including quarantine regulations, without in-depth knowledge of those pathogenic forms already present in a given area and others which may be a serious problem if introduced. Accurate diagnosis is essential, at species level and below, if pathogens are to be successfully detected and their presence and movement monitored. The ability to identify host specific strains of the fungus would be of obvious benefit in enabling in-depth epidemiological and ecological studies of pathogenic variability, leading to improved cultural and biological control and assisting in the selection and deployment of resistant material. Current work at IMI involves the characterisation, by a range of morphological, physiological, biochemical and molecular techniques, of key fungal pathogens including *F. oxysporum* (Bridge *et al.*, 1995, Rutherford *et al.*, 1994), *R. solani* (Bridge *et al.*, 1995) and *Colletotrichum* spp. (Waller *et al.*, 1993). This has resulted in the development of rapid laboratory techniques which will permit strain differentiation and identification of particular special forms of *F. oxysporum*, including *F. oxysporum* f.sp. *cubense*. Collaborative research programmes have now been initiated between scientists at IMI and at scientific centres in Uganda, Tanzania and Kenya, to facilitate the integration of these technologies into local research programmes to investigate the extent and severity of, and pathogenic forms responsible for, fusarium wilt of banana. Results of recent diagnostic surveys of banana-growing areas in these countries, in which a wide variety of banana cultivars are grown, have identified the disease as a major constraint to banana production (Gold *et al.*,

1993, Kung'u & Rutherford, 1996). Although knowledge of the world-wide variability of the pathogen already exists, it is known that Race 4 (and possibly other pathotypes of *F. oxysporum*) can overcome resistance exhibited by many of the banana and plantain cultivars commonly grown in these areas. Although sanitation measures can slow the spread of the disease, the most effective, economic and practical long term option for small farmers to combat the disease is to use disease-resistant plant material. Identification and development of locally adaptable germplasm with resistance to fusarium wilt is therefore considered critical. By enabling accurate identification of pathogen species or races, the pool of banana germplasm existing in farms and at research stations can be screened with confidence for resistance.

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FUSARIUM ROOT ROT OF PEAS

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ABSTRACT

Fusarium root rot of peas is an important root pathogen worldwide. Pea seedlings are first attacked at the cotyledonary attachment area, below ground epicotyl, and upper taproot. Any conditions which decrease root growth, including compaction and excessive temperatures, will increase *Fusarium* severity. Tillage pans formed from over 50 years of farming in the Pacific Northwest have created a soil environment favorable for fusarium root rot development. Even though viable and virulent inoculum of *F. solani* f. sp. *pisi* can be found as deep as 60 cm in the soil profile, infection of the upper 20 cm of a pea root system is necessary for severe disease development. Under field conditions, the reduction of soil inoculum of *F. solani* f. sp. *pisi* and soil compaction decreased root disease severity, and increased root length and dry seed yields. Progress has been made in developing resistance to fusarium root rot under both greenhouse and field conditions. Because fusarium root rot resistance can be reduced by an adverse environment or poor seed vigor, an integrated control is needed which includes both cultural practices and genetic resistance.

SYMPTOMS AND PATHOGEN

Fusarium root rot of peas (*Pisum sativum*), caused by *Fusarium solani* f. sp. *pisi,* is a serious root pathogen wherever peas have been grown commercially (Kraft *et al.,* 1981). Above ground symptoms consist of stunted growth and yellowing of the basal foliage. Initial symptoms on seedling roots consist of reddish-brown to blackish-brown streaks which can coalesce. A red discoloration of the vascular system can occur in the tap root but usually does not progress above the soil line.

In culture, *F. solani* f. sp. *pisi* produces blue-green to buff coloured sporodochia. Macrocondidia are primarily 3-septate, 4.4 to 5 µm by 27 to 49 µm, curved, and hyaline. Microconidia are less abundant, except in liquid culture where they are numerous. Chlamydospores, produced in the mycelium or by conidial conversion, are abundant, intercalary, terminal, single, or in chains. The teleomorph, *Nectria haematococca* syn.: *Hypomyces solani,* was reported to occur on diseased mulberry (*Morus* sp.) in Japan. Matuo & Snyder (1972) reported that *F. solani* f. sp. *pisi* was identical with the pathogen causing blight of mulberry and root rot of ginseng (*Panax* sp.). Distinct heterothallic isolates of *F. solani* f. sp. *pisi* exist and do not intercross but homothallic isolates also exist. This pathogen can be isolated from infected plant material and field soil on acidified potato dextrose agar or by use of a *Fusarium* selective medium such as Nash & Snyder's PCNB medium (Nash & Snyder, 1962).

The initial centre of attack of pea seedlings by *F. solani* f. sp. *pisi* is the cotyledonary attachment area, below ground epicotyl, and upper taproot, and penetration often occurs through stomates on the epicotyl (Bywater, 1959). Infection can then extend upward to the soil line and downward into the root zone (Fig. 1). Cutinase has been proposed as necessary for *F. solani* f. sp. *pisi* to penetrate the surface of the below ground hypocotyl to establish infection (Koller *et al.*, 1982). Cutinase was detected at the penetration site where germinating conidia penetrated etiolated pea stems and inhibition of cutinase formation reduced virulence on pea stems (Shayikh *et al.*, 1977). However, when the wild-type strain of *F. solani* f. sp. *pisi* was compared to transformants lacking the ability to form cutinase in artificially infested soil, no differences in virulence to pea seedling roots were observed (Stahl & Schafer, 1992).

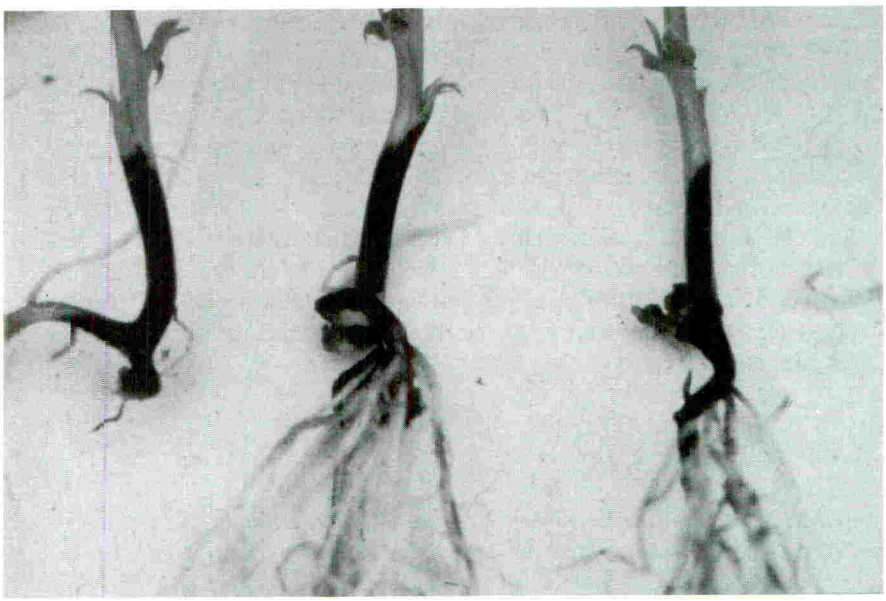

Figure 1. Typical symptoms of fusarium root rot of peas showing initial center of attack at cotyledonary attachment area.

The degree of root infection and damage is dependent upon the amount of stress the plant is exposed to. Any conditions which decrease root growth, including soil compaction, soil temperatures exceeding 30°C, soil moisture contents of -0.5 to -1.2 Mpa, soil pH (lower than 5.1 or higher than 7.5), and poor soil fertility will increase fusarium root rot severity (Kraft *et al.*, 1981; Allmaras *et al.*, 1988; Kraft *et al.*, 1988). Chlamydospores of *F. solani* f. sp. *pisi* are thought to germinate and produce pre-infection growth when stimulated by root and seed exudates. Rhizosphere effects may extend no more than 2 mm from the root surface into the surrounding soil and chlamydospore mobility is nil. Exudation from healthy pea roots is greatest near the root tip and along the zone of maturation (Huisman, 1982). *Fusarium* chlamydospores require 6 to 10 hours for germination, and growth toward a substrate would probably miss the root tip and make contact with the zone of maturation where exudation is

much less. Poor aeration and/or soil compaction can reduce root growth and may induce lateral root branching closer to the root apex, thus enhancing the probability that the germinating chlamydospore will make contact with the root tip and the exudation zone. *Fusarium solani* f. sp. *pisi* tends to produce primary disease symptoms in the region of cotyledonary attachment, epicotyl, and hypocotyl, which are stationary and exude nutrients into the surrounding soil via seed exudates. It has been our experience that only when the entire root system is invaded does *F. solani* f. sp. *pisi* cause serious disease losses.

INCIDENCE AND CONTROL IN THE PACIFIC NORTHWEST

In eastern Washington and northeastern Oregon, peas are grown primarily in rotation with fall-planted cereals where rainfall adequately supports annual cropping. Processing pea yields have remained static over the last 50 years in contrast to wheat yields which have nearly tripled. The most important yield constraint of peas is root diseases caused by *F. solani* f. sp. *pisi*, *Pythium ultimum*, and more recently *Aphanomyces euteiches*. In this area, we have found a definite tillage pan in all pea, wheat, or wheat-fallow sites sampled regardless of soil type and whether dryland or irrigated. This tillage pan is approximately 20 cm deep and usually about 7 cm thick. In addition, the straw turned down after a wheat crop (mouldboard plough) was still present and largely undecomposed during the succeeding pea crop (Kraft & Allmaras, 1983). Estimates of the amount of incorporated straw ranges from 2000 to 7000 kg/ha. The tillage pan is significant because compaction directly affects the extent and severity of root disease. In a typical soil profile, *P. ultimum* was found in the upper 20 cm and was absent below the plough layer (Fig. 2). *Fusarium solani* f. sp. *pisi* propagules were

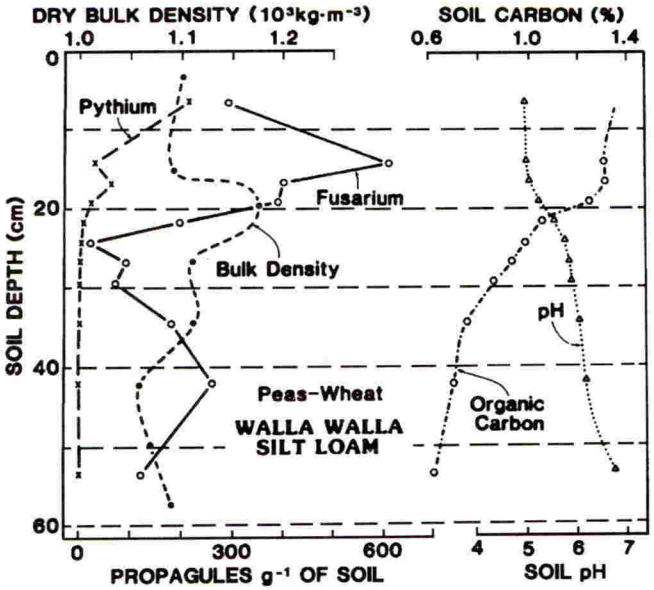

Figure 2. Typical relationship between *F. solani* f. sp. *pisi, Pythium ultimum* populations,and related soil properties indicative of long-term and recent tillage practices in a Walla Walla silt loam (pea-winter wheat rotation).

found throughout the upper 60 cm of soil, but their frequency was always low in the tillage pan under the plough layer. The low population of *F. solani* f. sp. *pisi* propagules in the tillage pan and their presence below it are probably related to impaired drainage from compaction in the tillage pan and the saprophytic survival of *F. solani* f. sp. *pisi* under dry soil conditions. In fields not cropped to peas for five or more years, *F. solani* f. sp. *pisi* was not detected in the plough layer but was detected below it. Colonies recovered from below the tillage pan were typical of *F. solani* f. sp. *pisi* and were virulent to pea roots. Long-term cultivation in this area has apparently produced an environment beneath the plough layer that is favourable for survival of *F. solani* f. sp. *pisi.*

In a later study, Rush & Kraft (1986) found that pea roots became infected when *F. solani* f. sp. *pisi* inoculum was placed in the lower 10 cm of 30 cm containers, but no above ground disease symptoms were evident. An inoculum concentration of 5000 cfu per g of soil, placed in the lower 10 cm, failed to cause any measurable plant stress. When inoculum was placed in the upper 10 cm or mixed throughout the containers, plant top and root weights, and leaf area were all significantly less than control plants growing in uninfested soil. There was no significant difference between plant stress measurements whether inoculum was placed throughout the soil profile or in the upper 10 cm. Apparently, in the absence of other stress factors, inoculum of *F. solani* f. sp. *pisi* deep in the soil profile has little detrimental effect on pea growth and development up to anthesis, when the upper 20 cm of the root system is not infected. When fields were sampled where peas had not been planted for five or more years, *F. solani* f. sp. *pisi* could not be detected in the plough layer and there was a corresponding increase in yields. In these same fields, *F. solani* f. sp. *pisi* could be detected below the plough pan.

A follow-up, 2 year field study was conducted to determine the effects of soil compaction and inoculum levels of *F. solani* f. sp. *pisi* on pea root length, disease severity, plant biomass, and dry seed yields (Kraft & Wilkins, 1989). Inoculum levels of *F. solani* f. sp. *pisi* were significantly reduced by fumigation with methyl bromide at the 0 to 20 cm depth but were not reduced below 20 cm. Use of a paraplough treatment to reduce compaction increased root density over that obtained with conventional mouldboard ploughing. The combination of fumigation and paraplough tillage decreased root disease severity and increased root length, biomass, and dry seed yields (Table 1).

Table 1. Effect of tillage and fumigation on disease severity, root length, and dry seed yield.

Tillage	Disease[a] severity	Root length (cm)	Seed yield (g/plot)
Mouldboard	4.5	16.3	1028.0
Mouldboard fumigated	0.9		1080.0
Paraplough	4.5	22.6	1165.5
Paraplough fumigated	1.9		1270.0

[a]Length of roots found in the first 20 cm of soil.

Glyphosate is being used as a viable alternative to mechanical weed control in the winter wheat-green pea rotation of southeastern Washington and northeastern Oregon. In a laboratory study, pathogenicity of *F. solani* f. sp. *pisi* was not reduced nor was mycelial growth significantly decreased at glyphosate concentrations greater that 2 mM (Kawate *et al.*, 1992). However, conidial production increased. *Fusarium solani* f. sp. *pisi* probably would not be affected adversely by glyphosate under field conditions because glyphosate would be diluted by weed biomass and adsorbed by soil colloids. However, Kawate *et al.* also found that glyphosate stimulated proliferation of *F. solani* f. sp. *pisi* in the rhizosphere of some common weeds sprayed with it. Apparently, after exposure to glyphosate, the plant system released nutrients into the rhizosphere in sufficient quantities to stimulate *F. solani* f. sp. *pisi* to increase significantly in cfu/g of soil in the rhizosphere of these weeds.

Breeding for Resistance

A breeding programme to incorporate resistance to *F. solani* f. sp. *pisi* in peas has been underway at Prosser, WA, since 1967. We first developed a procedure using artificially infested soil at an inoculum level of 20,000 to 40,000 cfu/g. Typically, under field conditions where fusarium root rot is chronic, inoculum levels vary from 500 to 2500 cfu/g. When inoculum levels are high, disease symptoms appear and individual plants can be evaluated for resistance in 10 to 14 days (Kraft, 1975). Inoculum levels, even up to 100,000 cfu/g, did not render a resistant pea line susceptible, although the disease indices increased (Fig. 3). Good seed vigour is an important consideration in comparing one pea line with other for resistance to fusarium root rot. A line with poor seed vigour may appear susceptible to fusarium root rot

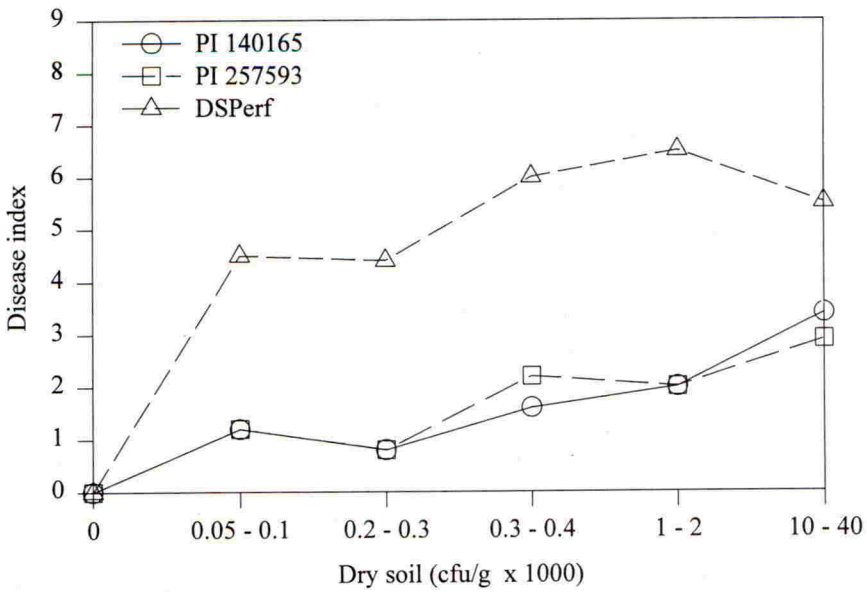

Figure 3. Effect of inoculum concentration on fusarium root rot severity.

when in fact it is quite resistant (Kraft, 1986). Pea seed and seedling exudates and extracts from testae of all Plant Introduction (PI) accessions tested with the A gene for anthocyanin production, whether resistant or susceptible to fusarium root rot, contained the anthocyanin (anthocyanin aglycone) pigment delphinidin. Delphinidin, located primarily in the testae of all PI accessions tested, was fungistatic to conidial germination of *Fusarium solani* f. sp. *pisi* (Kraft, 1977). However, in a bioassay, *F. solani* f. sp. *pisi* was able to germinate and grow when glucose was present in sufficient amounts. These results also pointed to the importance of seedling vigour when evaluating pea lines for resistance to fusarium root rot. In addition, PI accessions can be susceptible in the seedling stage despite the presence of delphinidin if they exude sufficient sugar. Because seed and seedling vigour is so important in the development of fusarium root rot, we are now using a seed soak test to screen peas for resistance to fusarium root rot (Kraft & Kaiser, 1993). Seeds of test lines are soaked overnight in a conidial suspension of *F. solani* f. sp. *pisi* adjusted to 1 X 10^6 per ml. Inoculated seeds are then planted into coarse-grade perlite in plastic flats and incubated in the greenhouse for 2 weeks. The resultant plants are scored for disease severity on a 0-5 scale where 0 = no disease and 5 = completely rotted root and/or seed rot. Lines that perform well in pure screening tests are then evaluated in a root disease field nursery infested with several root pathogens including *F. solani* f. sp. *pisi*.

CONCLUSIONS

Good progress has been made and will continue to be made in developing peas with acceptable horticultural attributes and inheritable resistance to fusarium root rot. Because resistance to fusarium root rot is not of a high level, we believe an integrated control approach is needed which includes cultural practices, maintenance of good seed vigour, and genetic resistance.

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NEW FINDINGS ON THE EPIDEMIOLOGY OF FUSARIUM EAR BLIGHT ON WHEAT AND ITS CONTROL WITH TEBUCONAZOLE

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ABSTRACT

Fusarium ear blight can produce considerable quantitative and qualitative damage in wheat. Investigations in Germany from 1987 to 1995 showed an increased incidence of *Fusarium* spp.. Epidemiological studies demonstrated the complexity of the biology of fusarium diseases and the difficulty of optimising the timing of fungicide applications. Nevertheless, products containing tebuconazole proved highly effective in terms of disease and mycotoxin reduction, yield increase and improved baking performance.

INTRODUCTION

Fusarium spp. are widespread pathogens in cereals and can attack crops from sowing to harvest. Tackling fusarium diseases in wheat is a complex problem. Current knowledge of the epidemiology of fusarium diseases is still deficient in many details and does not, at present, permit any clear prognoses for the optimal use of fungicides. Moreover, only a few highly effective active ingredients are available and optimal timing of these is vital, but difficult to achieve.

The importance and epidemiology of fusarium ear blight in Germany were investigated between 1987 and 1995. Results from France and Germany on the control of *Fusarium* spp. with products containing tebuconazole in terms of disease reduction, yield increase and improved grain quality are also presented in this paper.

MATERIALS AND METHODS

Investigations were performed on 100-grain samples from 326 wheat crops over 7 years (Figure 1), either taken from ears at Zadoks growth stage GS 85 (soft dough stage), or harvested crops. After superficial disinfection with NaOCl (2% available chlorine), grains were placed on potato dextrose agar and incubated under long-wave UV light for 7 days. The *Fusarium* spp. were identified microscopically after Nelson *et al.* (1983).

Over 3 years (1993 to 1995) maize-crop debris was checked for perithecial formation and their degree of ripening under natural infection conditions in a total of nine field trials. At one site, ascospore release 10 cm above the ground and at ear height of wheat was ascertained over 2 years with the aid of a spore trap (Suty & Mauler-Machnik, 1996)

In Germany, treatments with tebuconazole (as ®Folicur 250EW or ®Matador) were carried out once or twice starting at growth stage GS 55, under natural infection conditions. In 45 trials in France between 1986 and 1995, tebuconazole treatments were applied at different

times before or after the inoculation with *Fusarium* spp. Ears were inoculated at growth stage GS 65 by spraying (500 litres/ha) a conidial suspension (10^6 conidia/ml). There were four replicates of each programme arranged in a randomised 45 m^2 block design.

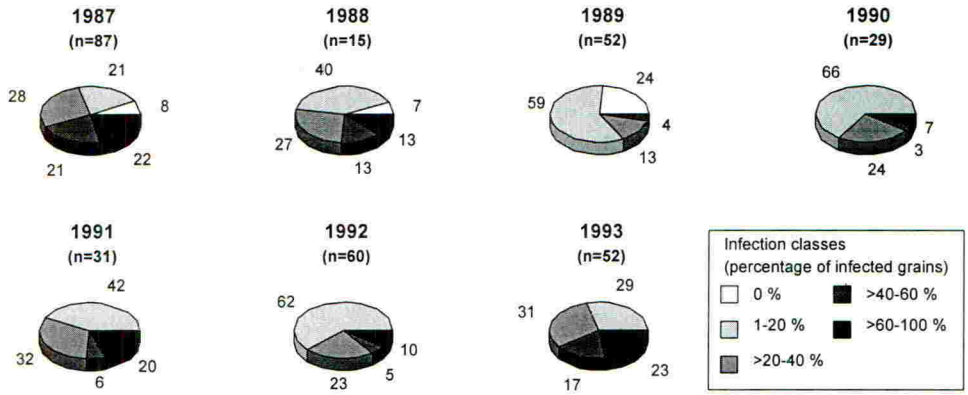
RESULTS AND DISCUSSION

Occurrence of *Fusarium* spp. in Germany

The five most important *Fusarium* spp. isolated from wheat ears in Germany are *F. graminearum*, *F. culmorum*, *Microdochium nivale* (syn. *F. nivale*), *F. avenaceum* and *F. poae*. However, the climatic requirements of the different species vary. An infection of *F. nivale* requires cold, damp conditions, whereas long warm and wet periods are needed for *F. graminearum* and *F. culmorum* (Mauler-Machnik & Zahn, 1994). The main time for ear infection is at full flowering (e.g. Strange & Smith, 1971). The co-occurrence of favourable temperature and humidity conditions (when wheat is flowering and disease pressure high) determines the intensity of ear infection.

Figure 1 shows the percentage of the investigated wheat fields in various infection classes between 1987 and 1993. Ear blight levels differed from year to year depending on the weather conditions. Infection levels were particularly high in 1987, 1991 and 1993. However, even in low pressure disease years, some fields showed a high infection level locally. Since 1990, all 172 investigated fields provided some grains infected with *Fusarium* spp.

Figure 1. Ear blight in wheat in Germany: percentage of the investigated wheat fields (n) in the various infection classes between 1987 and 1993.



Furthermore, investigations on the spectrum of *Fusarium* spp. showed that the dominant species may vary over several years depending on the climatic conditions at flowering. However, *F. graminearum* was the most common species isolated from wheat in Germany (Mauler-Machnik & Zahn, 1994). This species predominated not only in Bavaria as in the past, but was isolated from various wheat samples from central and northern Germany. The growing importance of *F. graminearum* may be related to the increased cultivation of maize in Germany. Independent of annual variations, a distinct increase in *F. poae* was demonstrated. Although this species does not play an important role in terms of yield, it

produces considerable amounts of mycotoxins and, for this reason, should continue to be investigated.

Significance of the teleomorph *Gibberella zeae*

F. graminearum was rarely isolated from leaves in our investigations and it appeared that ears could be infected without involvement of leaves. Since ascospores of *Gibberella zeae* (anamorph *F. graminearum*) can be transported over long distances by the wind (Reis, 1988), it was suspected that the teleomorph of *F. graminearum* could be involved in the epidemiology of ear blight caused by this fungus in Germany.

Perithecia on maize debris were formed in May between 1993 and 1995, in six of the nine field trials (Table 1); no perithecia were seen before May. According to Ye (1980), the formation of perithecia was promoted by high temperatures and wet weather. Numbers of perithecia remained generally low, as did ear infection levels. However, at the Dingolfing (a) location in 1994 perithecia were particularly abundant; here 52% of the maize debris produced perithecia and more than 2000 perithecia could be counted on one fragment with a surface area of 50 cm². This represents an inoculum potential of about 10^8 ascospores, given that a perithecium can contain up to 45000 ascospores (Kongha & Sutton, 1988).

Table 1. Formation of perithecia and dispersal of ascospores of *G. zeae* and infection of wheat ears at GS 85.

Rural district	Year	% of maize debris with perithecia	Number of perithecia *	Ascospore peak (number/cm² of slide)	% Infected spikelets
Main-Kinzig	1993	19	+	-	0
Main-Kinzig	1994	0	0	-	0
Dingolfing (a)	1993	30	+	-	-
Dingolfing (a)	1994	52	++++	18.9	27
Dingolfing (a)	1995	20	+	3.2	0
Dingolfing (b)	1993	22	+	-	1
Herford	1993	0	0	-	0
Herford	1994	0	0	-	0
Unstrut	1994	10	+	-	-

*+ = few; ++ = moderately abundant; +++ = abundant; ++++ = very abundant; - = not determined

At the Dingolfing location, ascospore dispersal was investigated in 1994 and 1995. In 1994 (Figure 2) ascospores were caught from the end of May to the end of June. In general the mean number of ascospores caught at ground level was approximately twice that at the wheat ear level. Two peaks of ascospore release at ear level were observed: at heading and at flowering. In each case, it rained shortly before or during ascospore release. Consequently, ear infection was high at GS 85 and reached 27% infected spikelets in the control plot. The number of ascospores caught during the same period in 1995 was very small and no clear ascospore peak was detected.

Effect of tebuconazole on ear blight and grain quality

In vitro studies with the aid of a germ tube test demonstrated that tebuconazole was similarly very effective against ascospores of *G. zeae* and conidia of *F. graminearum* (Suty & Mauler-Machnik, 1996). At the Dingolfing site, treatments with products containing tebuconazole

were carried out on the basis of the ascospore peaks observed (Figure 2). The two ascospore peaks at ear level showed clearly the difficulty of correctly timing fungicide applications against ear fusarioses. The efficacy achieved after a single treatment (250 g a.i./l) at GS 55 or GS 65 was about 50% (Figure 3). The increase of active ingredient (375 g a.i./l) at GS 65 improved the efficacy to 65%. On the other hand, a double application using reduced application rates (200 g a.i./l) increased efficacy to more than 70%.

Figure 2. Dispersal of *G. zeae* ascospores in the field, 1994.

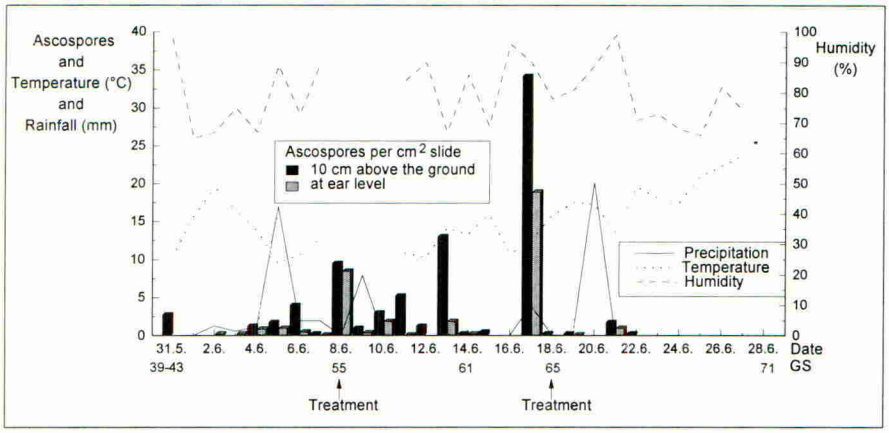
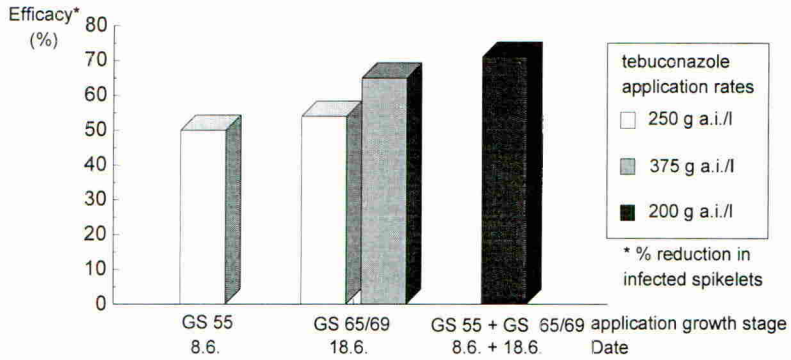


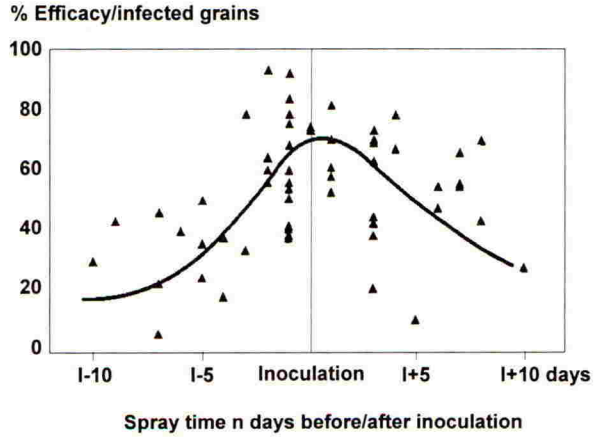
Figure 3. Efficacy of products containing tebuconazole in relation to ascospore peaks.



Numerous field trials inoculated with *F. graminearum* or *F. culmorum* were carried out in different countries in western Europe, and especially in France. Figure 4 shows the efficacy of products containing tebuconazole achieved after treatments at different times either side of the inoculation date. Efficacies averaging approximately 60% (range: 40 to 95%) were achieved after tebuconazole applications made within 4 days before or after inoculation. Efficacy was reduced (range: 30 to 50%) for treatments applied 5 to 10 days before or after inoculation. Treatments carried out 1 to 5 days after inoculation were slightly more effective than treatments applied 1 to 5 days before inoculation. However, this depended on the difference between inoculation and actual infection date.

Field studies showed that application technique has a direct effect on efficacy. Using normal spraying machinery only one ear face is sprayed. Analysis of tebuconazole contained in different parts of ears demonstrated that only a partial redistribution takes place, essentially from the ear rachis. This shows the necessity for improving spray cover on the ears and thus increasing the penetration of tebuconazole into the rachis. For example, the use of double fan nozzles, one spraying forwards and the other backwards, improved the efficacy of tebuconazole against ear fusarioses significantly (93% efficacy compared to 77% with a single standard spray nozzle) (Courbon, 1995).

Figure 4. Efficacy of products containing tebuconazole applied at different timings around the inoculation date.



In the case of ear blight, grain quality criteria are of particular importance. *Fusarium* spp. can not only affect grain constituents but can also produce their own metabolic products with toxic properties. *F. graminearum, F. culmorum* and *F. poae* can produce mycotoxins which are highly toxic to man and animals. Pontzen (1993) showed that after natural *Fusarium* infection treatments with products containing tebuconazole led to a clear decrease in deoxynivalenol content (Table 2). Similar results were obtained after artificial inoculation with *F. culmorum* (Mesterhazy & Bartók, 1996; Homdork *et al.*, 1996).

Table 2. Effect of ear treatment with products containing tebuconazole on the mycotoxin deoxynivalenol (DON) content of wheat grains (from Pontzen, 1993).

Treatment	Efficacy (%)	Relative yield (%)	DON (mg/litre)
Control	(55)	100	11.2
tebuconazole (250 g a.i./litre)	50	121	3.0

( ) = % of grains infected

In 1995, baking performance studies in France showed that the use of flour from crops infected with ear blight led to a distinct deterioration in baking performance. In spite of a high disease severity (33% infected grains), a single treatment with tebuconazole resulted in a clear

disease reduction and consequently a significant improvement in baking quality in terms of dough texture, bread volume and knead capacity (Courbon, 1995).

CONCLUSIONS

Despite annual variations in disease severity, the incidence of ear blight, and especially *F. graminearum,* has increased in Germany in the last few years. Fusarium infections of ears can cause considerable damage in terms of yield and quality. Investigations on the teleomorph *Gibberella zeae* showed the difficulty of correctly timing fungicide applications. However, products containing tebuconazole showed a high level of efficacy against ear blight when applied within a few days of inoculation - particularly if the spray covered more than one side of the ears. Their utilisation leads to a disease reduction, a clear yield increase and an improvement of quality in terms of baking performance and mycotoxin content. Since ear fusarioses are still very difficult to control, full use should be made of agronomic measures (such as ploughing-in crop debris) in accordance with the principles of integrated crop protection.

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PRODUCTION AND CONTROL OF MYCOTOXINS FROM *FUSARIUM* SPECIES PATHOGENIC ON CEREALS

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ABSTRACT

Several *Fusarium* spp. that are pathogens of cereals are also potential sources of mycotoxins which may contaminate the grain and prejudice human and animal health. Particular risk emanates from the trichothecenes, including T-2 toxin and deoxynivalenol and from the fumonisins. Deoxynivalenol concentrations in cereal grains have been correlated with the incidence and severity of fusarium ear blight. Recent field trials suggest a limited role for fungicides in the control of deoxynivalenol contamination of wheat grain. With T-2 toxin, there is evidence, from laboratory studies, of enhanced synthesis on exposure of *F. sporotrichioides* to certain fungicides. However, the use of cereal genotypes resistant to fusarium diseases offers good prospects for protection from mycotoxin contamination of grain.

INTRODUCTION

Fusarium spp. have long been associated with diseases of economically important fusarium plants. In particular, fusarium ear blight (scab) of wheat, barley and oats has been linked with over 15 species of this genus (Parry *et al.*, 1995). The most common species include: *Fusarium graminearum, F. culmorum, F. avenaceum, F. poae, Michrodochium nivale (F. nivale), F. sporotrichioides* and *F. oxysporum.* Of these species causing fusarium ear blight, *F. graminearum* and *F. culmorum* are considered to be the most pathogenic (Parry *et al.*, 1995). *F. graminearum* has also been implicated in crown rot of wheat (Wildermuth & McNamara, 1994). In maize, ear rot may occur as a result of infection with a number of *Fusarium* spp., including *F. graminearum* and *F. moniliforme* (Schaafsma *et al.*, 1993).

Although ear blight of cereal plants can result in severe losses in yield, an additional penalty may emanate from contamination of the harvested grain with *Fusarium* mycotoxins. These substances are secondary metabolites that are toxic to animals and humans consuming contaminated grain.

TOXIGENIC *FUSARIUM* PHYTOPATHOGENS

Fusarium spp. of fungi produce a wide range of mycotoxins, of which the most significant from the standpoint of human and animal health are the trichothecenes, zearalenone and its derivatives, fusaric acid and the fumonisins (D'Mello *et al.*, 1996a). In excess of 100 trichothecenes have been isolated, characterised and classified into four types. However, most attention has focused on Type A trichothecenes, including T-2 toxin, HT-2 toxin, neosolaniol (NEO) and diacetoxyscirpenol (DAS) and on Type B trichothecenes, comprising nivalenol (NIV), deoxynivalenol (DON; vomitoxin) and its 3-acetyl and 15-acetyl derivatives

(3-ADON and 15-ADON, respectively). The structures of the major trichothecenes and other *Fusarium* mycotoxins are presented by Flannigan (1991).

The production of mycotoxins by common phytopathogenic *Fusarium* spp. is summarised in Table 1, compiled from investigations over the past 20 years or so (D'Mello *et al.*, 1996a). The table is not exhaustive but rather illustrative of certain distinguishing features in the production

Table 1. Production of *Fusarium* mycotoxins.

Fusarium species	Mycotoxins
F. sporotrichioides; F. poae	T-2 toxin, HT-2 toxin, neosolaniol, diacetoxyscirpenol
F. poae	Nivalenol
F. graminearum; F. culmorum	Deoxynivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol
F. avenaceum	Deoxynivalenol, 15-acetyl deoxynivalenol
F. sporotrichioides; F. graminearum; F. culmorum	Zearalenone
F. oxysporum	Zearalenone, fusaric acid
F. moniliforme	Fumonisins

of the commonly occurring mycotoxins of this genus. It is clear, for example, that the production of Type A trichothecenes predominates in *F. sporotrichioides* and *F. poae*, although the evidence is less convincing for the latter species (D'Mello *et al.*, 1996a). Production of Type B tricho-thecenes occurs principally in *F. culmorum* and *F. graminearum*. However, there is much evidence to suggest that *F. poae* is also a Type B trichothecene producer. A notable feature is that many *Fusarium* spp. have the capacity to synthesise other mycotoxins, in addition to the trichothecenes. Thus, zearalenone (ZEN) is produced by three of the species previously mentioned as well as by *F. oxysporum* which also synthesises fusaric acid. *F. moniliforme* is a well-recognised source of the fumonisins which comprise six structurally related metabolites. Of these, fumonisins B_1 and B_2 (FB_1 and FB_2 respectively) have been implicated in human and animal disorders (D'Mello & Macdonald, 1996; D'Mello *et al.*, 1996a).

The classical assessment of toxicity of different deleterious substances centres on the determination of LD_{50} values in experimental animals. Flannigan (1991) lists LD_{50} values of 4.1, 5.2, 9.0, 14.5, 23.0 and 70.0 mg/kg body weight for NIV, T-2 toxin, HT-2 toxin, NEO, DAS and DON, respectively when these mycotoxins were administered intraperitoneally to mice. Although the acute toxicity of DON is relatively low, it is widely recognised as a potent

feed intake inhibitor in pigs which accounts for its alternative name, vomitoxin. Zearalenone is even less toxic in the classical sense, with LD_{50} values for different animals ranging from 2 to 10 g/kg (Flannigan, 1991), but it has been associated with infertility, reduced milk production and hyperoestrogenism in cows (see D'Mello *et al.*, 1996a). Fusaric acid appears to be of minor toxicity at levels detected in nature, but there is increasing evidence that it may act synergistically to enhance the activity of other *Fusarium* mycotoxins. The fumonisins are associated with diverse manifestations of toxicity in farm animals, but these mycotoxins have recently been linked with the incidence of oesophageal cancer in humans (Yoshizawa *et al.*, 1994).

FUSARIUM DISEASES AND MYCOTOXIN PRODUCTION IN CEREAL GRAINS

Owing to the ubiquitous occurrence of DON in cereal grains, considerable work has been conducted to elucidate its relationship with fusarium ear blight. In one such study (Miller *et al.*, 1985), a single isolate of *F. graminearum* was used to infect different cultivars of spring wheat, rye and triticale. Resistant cultivars of the three cereals contained low concentrations of DON (mean 0.6 mg/kg) in the kernels whereas grain from susceptible cultivars had considerably higher concentrations (mean 10.2 mg/kg) despite minimal visual evidence of ear blight in the plants. However, in a subsequent study, pathogenicity in field trials was correlated with mycotoxin contamination of wheat grains (Wong *et al.*, 1995). Thus in that study, *F. culmorum* and *F. graminearum* were found to exhibit the greatest pathogenicity in comparison with *F. sporotrichioides* or *F. avenaceum*. Correspondingly, higher concentrations of DON were detected in grain of susceptible wheat cultivars than in grain from resistant cultivars after inoculation with the two most pathogenic *Fusarium* spp. For example, in susceptible cultivars inoculated with *F. culmorum,* DON values ranged from 17 to 121 mg/kg grain whereas in resistant cultivars concentrations of 0.2 to 9.7 mg/kg grain were recorded. In two of these susceptible cultivars, 15-ADON levels ranged from 0.11 to 0.21 mg/kg, but in all resistant cultivars levels of 15-ADON were below the detection limit. In a more recent investigation, with winter wheat, mean concentrations of DON were positively correlated with both ear blight incidence and severity in natural epidemics (Wiersma *et al.*, 1996). Within *F. culmorum*, strains differing in pathogenicity have been found (Snijders & Perkowski, 1990). In plot trials with experimental inoculations, the most virulent strain induced the highest incidence of ear blight in wheat, particularly in susceptible cultivars, and there was close correlation between ear blight incidence and contamination of kernels with DON. None of the other trichothecenes or ZEN was detected in any of the wheat grain samples.

In maize, ear rot has also been attributed to infection with *Fusarium* spp., particularly *F. graminearum*. Field trials with artificial inoculations indicated that ear rot severity was greater with *F. graminearum* than with *F. moniliforme* or *F. subglutinans* (Schaafsma *et al.*, 1993). Ear rot severity generally correlated well with levels of DON and the authors concluded that severity of ear rot may be a useful indicator of mycotoxin production in grain.

It is important to recognise that in the field and at harvest, grain is likely to be colonised by different species of fungi (D'Mello *et al.*, 1993) and the potential for mycotoxin production may be influenced by fungal interactions. Cuero *et al.* (1988), for example, showed that ZEN production was markedly decreased at 16°C by the presence of *A. flavus* but remained unaffected at 25°C.

CONTROL MEASURES

It is axiomatic that preventive measures are of paramount importance in reducing the risk of mycotoxin contamination of cereal grains. Two obvious strategies may be envisaged, both involving the prevention of fungal proliferation and disease. As might be anticipated, fungicides can influence mycotoxin production but the effects are variable and dose-dependent (D'Mello *et al.*, 1996a). In laboratory experiments with pure cultures (Table 2) dicloran, iprodione and vinclozolin were individually effective as inhibitors of DAS and ZEN synthesis in *F. graminearum* but tridemorph and carbendazim each enhanced T-2 toxin production in *F. sporotrichioides*, while 3-ADON production increased in *F. culmorum* treated with difenoconazole.

Table 2. Effects of fungicides on production of mycotoxins in pure cultures of *F. graminearum*, *F. sporotrichioides* and *F. culmorum*.

Fungicide	Methods	Effects	Ref.
Dicloran, iprodione, vinclozolin	Added separately at levels of up to 500 µg/ml potato-dextrose broth; static culture of *F. graminearum*	Dose-related inhibition of growth and production of DAS and ZEN; total inhibition of DAS and ZEN production at higher levels of each fungicide	1
Tridemorph	Shake-flask cultures of *F. sporotrichioides*; fungicide added at 6 and 36 µg/ml	At 6 µg/ml, growth enhanced but T-2 toxin and DAS production inhibited; at 36 µg/ml, growth inhibited but T-2 toxin production stimulated	2
Carbendazim	Cultures of *F. sporotrichioides* on potato-dextrose agar; fungicide added at 5 µg/ml	Growth unaffected by fungicide; significant increase in T-2 toxin production with fungicide	3
Difenoconazole	Cultures of *F. culmorum* on potato-dextrose agar; fungicide added at levels of up to 100 µg/ml	Growth unaffected by 0.1 µg/ml but 3-ADON production signifi-cantly increased	4

Refs: 1. Hasan (1993); 2. Moss & Frank (1985); 3. Placinta *et al.* (1996); 4. D'Mello *et al.* (1996b).

Field trials with fungicides have yielded somewhat conflicting results. Thus Boyacioglu *et al.* (1992) showed that propiconazole reduced infection of wheat by an artificially applied inoculum of *F. graminearum* by 39-55% and DON levels were reduced by 34-78%. However, thiabendazole had no effect on infection level but DON contamination was reduced

by up to 83%. In a subsequent study, also with wheat inoculated with *F. graminearum* (Milus & Parsons, 1994), ear blight incidence and DON concentrations in grain remained unaffected by propiconazole, thiabendazole or tebuconazole applications. On the other hand, combination of tebuconazole and triadimenol in wheat inoculated with *F. culmorum* reduced ear blight but a 16-fold increase in NIV content of grain was observed (Gareis & Ceynowa, 1994).

It is a consistently held view that exploitation of genetic resistance to diseases such as ear blight offers the most promising method for control of mycotoxin contamination of cereal grains. Two elegant studies reinforce this concept. Thus Snijders & Perkowski (1990) showed that in wheat genotypes resistant to ear blight caused by *F. culmorum*, DON concentrations of grain varied from 3.4 to 4.6 mg/kg. However, in susceptible genotypes DON levels increased to 37 mg/kg. Subsequently, Wong *et al.* (1995) demonstrated that several Chinese cultivars of wheat were resistant to ear blight induced by either *F. culmorum* or *F. graminearum* and DON contamination of grain was low, with a maximum value of 9.7 mg/kg. In contrast, three Canadian cultivars susceptible to ear blight had concentrations of up to 121 mg DON/kg kernel.

CONCLUSIONS

Fusarium spp. are significant not only as phytopathogens but also as potential producers of mycotoxins which may prejudice safety of food, particularly cereal grains. The trichothecenes, including T-2 toxin and deoxynivalenol, and the fumonisins are the most important mycotoxins in this context. Several studies suggest good correlation between fusarium ear blight of cereals and deoxynivalenol contamination of grain. Recent field trials have not provided a consensus regarding the efficacy of fungicides to control mycotoxin production. Indeed, laboratory studies consistently indicate that T-2 toxin may be enhanced with applications of certain fungicides. The exploitation of disease-resistant cereal genotypes, however, represents a promising strategy for reducing mycotoxin contamination of grain. Studies are being undertaken at the Scottish Agricultural College to assess how mycotoxin production may be affected in fungicide-resistant strains of *Fusarium* spp.

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