SESSION 2A

NEW COMPOUNDS AND USES FOR PEST MANAGEMENT

Chairman & Session Organiser: Dr R Bateman
CABI Bioscience, Ascot, UK

Papers: 2A-1 to 2A-7

Insect neuropeptide fusion proteins - a new generation of orally active insect control agents

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ABSTRACT

Neuropeptides regulate many aspects of insect physiology, and have potential as insect control agents. Neuropeptide pesticides potentially offer levels of activity, specificity and environmental compatibility, absent in conventional insecticides. However, neuropeptides are generally poor candidate insecticides, because they do not easily penetrate the cuticle, and degrade rapidly in the environment and insect gut. *Manduca sexta* allatostatin (Manse-AS), regulates juvenile hormone biosynthesis in moths, and has myoregulatory action on the gut. Moreover, Manse-AS produces marked reductions in feeding and growth, when injected into larvae of the Tomato moth. The Snowdrop lectin (GNA) is detectable in the haemolymph of larvae following oral administration. To determine whether GNA could transport neuropeptides across the gut, a recombinant expression system was used to produce a GNA/Manse-AS fusion protein (FP). Following expression in *E. coli*, the purified FP was incorporated into artificial diet and offered to Tomato moth larvae. The intact FP appears in the haemolymph following oral administration, and results in an almost total cessation of feeding and growth by larvae exposed to FP diet. These results offer the possibility of developing a whole range of novel, orally-active, target-specific, pesticides based on insect neuropeptides. In this paper, the nature and potential of such novel pesticides is discussed.

INTRODUCTION

Many aspects of insect physiology are controlled by peptide hormones (Raabe, 1989; Holman, 1990; Joosse & Geraerts, 1990) and the possible deployment of peptide hormones or their analogues as novel insecticides has been the subject of much recent investigation and speculation (Menn *et al.*, 1989; Keeley *et al.*, 1990; Kelly *et al.*, 1990; Masler *et al.*, 1993; Nachman *et al.*, 1993 Hoffmann & Lorenz, 1998; Weaver *et al.*, 1998). However, insect neuropeptides have a number of characteristics that, at first sight, make them appear rather unsuitable candidate insect control agents. In general, these molecules are likely to be unstable in the environment, suffer rapid degradation in the digestive systems of target species and, be relatively ineffective in penetrating the insect cuticle. Consequently, considerable effort has been directed to the discovery and development of effective delivery systems. In particular, studies have concentrated on the possible use of genetically modified baculoviruses as vectors for genes expressing neuropeptides and other insecticidal proteins (Maeda 1989; Wood & Granados 1991; Eldridge *et al.*, 1992).

We have adopted a rather different approach based on our previous studies with the snowdrop lectin (*Galanthus nivalis* agglutinin; GNA). This is one of several plant proteins that have been extensively examined with a view to improving pest resistance in crops by genetic modification (e.g. Fitches *et al.*, 1997; Gatehouse *et al.*, 1997). In earlier studies with GNA we discovered that this lectin was detectable in the haemolymph of larval *Lacanobia oleracea*, following oral ingestion of the lectin admixed with artificial diet (Fitches *et al.*, 2001). These observations led us to consider the possibility that GNA could be used to transport other proteins or peptides from the insect gut into the haemolymph.

We have also been investigating the potential of several insect neuropeptides as insect control agents. One such molecule, the neuropeptide allatostatin, Manse-AS (pE-V-R-F-R-Q-C-Y-F-N-P-I-S-C-F-OH) which was originally identified in the Tobacco hornworm *Manduca sexta* (Kramer *et al.*, 1991), appears to be present in several other lepidopterans (Weaver *et al.*, 1998). In larvae of *L. oleracea* this peptide does inhibit the biosynthesis of juvenile hormones (Audsley *et al.*, 1999), but may have additional, myoregulatory roles, including the control of gut peristalsis (Duve *et al.*, 2000). Moreover, injection of Manse-AS into the haemolymph of 5th stadium *L. oleracea* larvae has been shown to result in reduced feeding, retarded growth, and increased mortality (Audsley *et al.*, 2001a).

With these results in mind, we investigated whether GNA could be used to transport Manse-AS into the insect haemolymph, when a recombinant fusion protein combining these molecules was incorporated in larval food.

MATERIALS AND METHODS

Cloning, expression and purification of GNA and FP constructs

Constructs encoding either mature GNA, or a fusion protein (FP) in which the Manse-AS peptide was fused to the C-terminal of GNA *via* a 4 amino acid linker peptide were prepared, cloned and expressed in *E. coli* as described in detail by Fitches *et al.* (2002). Recombinant GNA and FP were purified, and Western analysis confirmed that both proteins reacted positively with anti-GNA antibodies, and that FP also reacted with anti-Manse-AS antibodies (Fitches *et al.*, 2002). Subsequently, the proteins were renatured by dialysis, and agglutination values for both recombinant proteins indicated that the C-terminal residues encoding the linker peptide and Manse-AS did not interfere with GNA functionality Fitches *et al.* (2002). A diagrammatic representation of the GNA-Manse-AS fusion protein is shown in Figure 1.

Insect bioassays: effects of FP on food consumption and growth

A potato leaf based artificial diet was used to assay recombinant proteins and various control materials (see below) against newly moulted 5th stadium *L. oleracea* larvae (see Fitches *et al.*, 2002). Larval wet weights (± 0.1 mg) were recorded before, during, and after exposure to the treatments, and diet consumption was estimated on a wet weight basis. The amounts of recombinant proteins added to diets were based on activity values derived from agglutination assays. Controls containing small amounts of either ammonia (equivalent to that used to solubilise recombinant proteins after freeze-drying) or methanol (equivalent to that used to dissolve Manse-AS) were tested in addition to a normal artificial diet control.

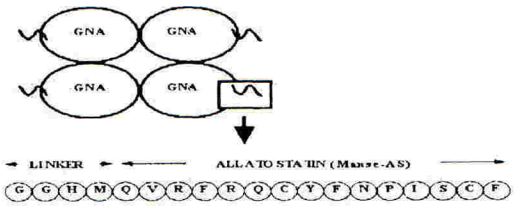

Figure 1.Diagramatic representation of FP, showing tetrameric GNA subunits, to each of which Manse-AS is fused via a four amino acid linker. The amino acid sequence of the linker and of Manse-AS is denoted. Full sequence data is given in Fitches, *et al.* (2002).

Haemolymph collection, HPLC fractionation, and immunoassay.

In order to investigate the putative transport of Manse-AS by the FP, aliquots of haemolymph from larvae fed various diets were purified, and analysed for the presence of Manse-AS-like immunoreactivity by indirect ELISA (Audsley *et al.*, 1998). Haemolymph was extracted from insects fed for 48 h on diet containing FP (at a concentration of approx. 0.1 % (w/w) of dietary protein), or on various control diets. Samples were extracted and prepared as described by Fitches *et al.* (2002).

RESULTS

Insect Bioassays: effects of FP on food consumption and growth.

When artificial diet containing either FP at 0.5 % of dietary protein, or containing a variety of control materials (see above), was offered to 5th stadium larvae of the Tomato moth, *Lacanobia oleracea* for 3 days to diets containing. The results (Table 1) showed that insects fed on control (untreated) diet, or on diet containing native GNA, or on a diet containing a combination of native GNA and synthetic Manse-AS, showed a fourfold increase in weight during the assay period (Fitches *et al.*, 2002). By marked contrast with the results obtained with the various control diets, all larvae exposed to diet containing FP exhibited a significant ($p< 0.0001$; ANOVA) reduction in mean weight over the assay period (Table 1).
Small differences in larval weight gain were seen with diet containing either GNA or Manse-AS (Table 1). However, similar reductions were apparent in the control diets containing ammonia or methanol (used to increase solubility of FP and Manse-AS, respectively), indicating that these effects were largely due to the solvents. The substantial, negative effects on weight gain by larvae offered FP diet was reflected by their minimal consumption of this diet by comparison with control diets (Table 1). In fact, evidence of feeding by FP-exposed larvae was apparent only by the presence of small quantities of diet in dissected larval guts.

Table 1. Mean increase/decrease in weight of *L. oleracea* larvae (corrected for larval weight at time 0) and mean consumption (g wet weight artificial diet) following exposure to control diet; control diet + ammonia, control diet + methanol, Manse-AS (0.5 mg/5g diet), GNA (0.5 mg/5g diet), GNA + Manse-AS (0.5 mg/5g diet) or recombinant FP (0.5 mg/5g diet) for 3 days. Data shows means ± SE (n=8). ANOVA column gives significance of difference, (A) between treatment and control + ammonia (control for diet containing FP), and (B) between Manse-AS treatment and control + methanol (control for Manse-AS treatment). Data based on Fitches *et al.*, 2002).

Treatment	Increase/decrease in larval weight	ANOVA	Diet consumption (g)	ANOVA
Control	3.27 ± 0.0061	(A) 0.0078	0.508 ± 0.0144	(A) NS
Control + ammonia	2.83 ± 0.0057	A	0.470 ± 0.0386	A
Control + methanol	1.58 ± 0.0048	B	0.408 ± 0.0145	B
Manse-AS	1.65 ± 0.0060	(B) NS	0.414 ± 0.0176	(B) NS
GNA	2.92 ± 0.0037	(A) NS	0.415 ± 0.0195	(A) NS
GNA + Manse-AS	3.16 ± 0.0048	(A) NS	0.392 ± 0.0175	(A) NS
FP	- 0.12 ± 0.0019	(A) <0.0001	0.0	(A) <0.0001

Haemolymph collection, HPLC fractionation, and immunoassay.

Haemolymph was extracted from insects fed for 48 h on diet containing FP (at a concentration of approx. 0.1 % (w/w) of dietary protein), or on various control diets. In these experiments, material reacting with anti-Manse-AS was present in blood samples taken from all larvae, irrespective of diet (Fitches *et al.*, 2002). However, quantitative indirect ELISA showed that these samples fell into two clearly defined groups. Haemolymph from insects fed on control diet, or diets containing native GNA, or somatostatin (a control peptide), had low levels of Manse-AS-like immunoreactivity (65.1 ±7.1, 59.6 ±7.3 and 68.8 ±3.6 fmol/50ug protein, respectively (n=4)). By contrast, pooled haemolymph from insects fed on diets containing either synthetic Manse-AS, or a mixture of GNA and Manse-AS, or FP, contained significantly higher levels of Manse-AS-like immunoreactivity (114.2 ±11.1, 104.8 ±6.9 and 128.3± 12.2 fmol/50ug protein, respectively ($p< 0.0001$ ANOVA; n=4)). Investigation of the nature of the material detected in haemolymph revealed that the major Manse-AS-immunoreactive fraction from the blood of FP-fed insects corresponded with the elution volume of an intact FP standard (Fitches *et al.*, 2002). By contrast, in all other samples, Manse-AS-like immunoreactivity was associated only with those fractions co-eluting with synthetic Manse-AS. These results strongly suggest that the FP was present as an intact molecule in the haemolymph of insects fed on diet containing this protein.

DISCUSSION

Using a novel recombinant fusion protein combining the snowdrop lectin (GNA) and an insect neuropeptide allatostatin (Manse-AS), we have shown that GNA can be utilised to transport the linked neuropeptide to the haemolymph of a lepidopteran larva following oral administration. In addition, this FP has been shown to have significant and deleterious effects upon the feeding and growth of larvae exposed to artificial diet containing the fusion protein. The negligible consumption of diets containing FP suggests that the observed effects were primarily due to some antifeedant action of the peptide.

The antibody used in these experiments would have detected both endogenous and exogenous antigen. Thus, the elevated levels of immunoreactivity observed in insects fed on the diet containing synthetic Manse-AS, could either be a direct result of transport of this peptide (or of an immunoreactive portion thereof) across the gut wall, or a result of endogenous allatostatin levels being increased by some other (unknown) mechanism. Nevertheless, since the diet containing Manse-AS alone did not have deleterious effects on insect growth or feeding, our results show that the effect of FP is fundamentally different to any effect produced by oral administration of the free peptide. The passage of FP from the gut to the haemolymph was indicated by the presence of high levels of Manse-AS-like immunoreactivity in haemolymph. Since the indicated molecular weight of this immunoreactive material was similar to that of an FP standard (Fitches *et al.*, 2002), it may be concluded that FP can cross the gut wall as an intact protein.

The mechanism(s) by which dietary FP affects *L. oleracea* larvae remains unclear. Although GNA is toxic to some insects, it has only relatively small effects on growth and development in this species (Fitches *et al.*, 1997; Fitches, 1999; Gatehouse *et al.*, 1997) and, as shown here, feeding native or recombinant GNA to *L. oleracea* larvae did not produce noticeable antifeedant or growth-retardant effects. Similarly, dietary administration of synthetic Manse-AS alone produced no observable effects on growth or feeding. However, evidence from recent studies suggests that this neuropeptide may indeed be involved in the regulation of feeding. In *L. oleracea,* Manse-AS-like immunoreactivity is present in neurones of the frontal ganglion, and in the axons that innervate the muscles of the foregut, and the peptide has been shown to have a reversible effect on myogenic contractions of the foregut *in vitro* (Audsley *et al.*, 2000; Duve *et al.*, 2000; Audsley *et al.*, 2001a). In addition, direct injection of Manse-AS into the haemolymph of *L. oleracea* larvae produced a significant reduction in feeding (Audsley *et al.*, 2001a). Interestingly, injection of insect sulfakinin peptides resulted in reduced feeding in locusts (Wei *et al.*, 2000), and other neuropeptides (leucokinins) inhibit the *in vitro* release of digestive enzymes by midgut preparations from the moth *Opisina arenosella* (Harshini *et al.*, 2002). Whether the effects observed with the GNA-Manse-AS fusion protein are mediated by similar mechanisms, or by some entirely novel action, remains a matter for speculation. However, the discovery (Duve *et al.*, 2000) that allatostatin-like immunoreactivity is detectable in the stomatogastric nervous system (especially the foregut and stomodeal valve), coupled with the fact that injection of the native peptide produces a marked reduction in foregut peristalsis (Audsley *et al.*, 2001a), strongly suggests that the action of FP is mediated through inhibition of normal gut movement.

We have shown previously that, injection of the Manse-AS peptide into the haemocoel of *L. oleracea* results in marked suppression of feeding (Audsley *et al.*, 2001a), whereas oral administration of this peptide has no marked effects (Table 1). We have also shown that the half-life of this peptide in *L. oleracea* haemolymph is very short (approx. 3.5 min; Audsley *et al.*, 2001b). These observations suggest that orally administered native Manse-AS may be rendered inactive at some point during its passage from the gut to the haemolymph. However, the antifeedant effects of the FP observed here, and the identification of Manse-AS-like immunoreactivity in the haemolymph of insects exposed to FP in the diet, indicate that Manse-AS, is delivered to the blood by GNA in a biologically active form. Thus, we suggest that the fusion of Manse-AS to GNA somehow protects the Manse-AS peptide, allowing it to remain active following delivery to the haemolymph. Whatever the mode of action, there exists a clear potential for this material to exert significant insecticidal effects if the

antifeedant properties of the FP observed in this study were realised in field conditions. Furthermore, the FP technology reported here, may have additional applications for the delivery of other peptides to insect blood by oral administration. Finally, although we have used recombinant techniques to produce the FP, there is no reason why this (and other peptide fusion proteins) could be synthesised by conventional chemical methods, thus permitting practical deployment of these novel pesticides without recourse to the use of genetic modification.

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Pyridalyl: A novel insecticidal agent for controlling lepidopterous pests

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ABSTRACT

Pyridalyl (experimental code number: S-1812) is an insecticide belonging to a novel chemical class. The compound has high insecticidal activity against larvae of various lepidopterous insects and motile stages of thysanopterous insects. It shows excellent control of important lepidopterous pests on cotton and vegetables without any phytotoxicity concerns at rates between 83 and 300 g a.i./ha. The compound also controls insecticide-resistant strains of lepidopterous pests as well as susceptible strains. Pyridalyl shows low toxicity to various beneficial arthropods. It is expected to be a useful material for control of lepidopterous and thysanopterous pests in Integrated Pest Management (IPM) and insecticide resistance management programmes.

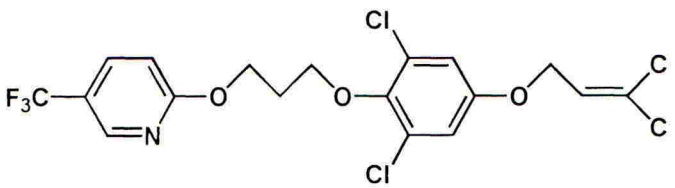
INTRODUCTION

In research to find new insecticidal molecules, we found out that 3,3-dihalo-2-propenyloxy phenyl derivatives have insecticidal activity (Sumitomo Chemical Co. Ltd., 1996). As a result of optimization, pyridalyl was selected as a promising insecticide for cotton and vegetables and is under development in Japan, United States, Europe and some countries (Sakamoto *et al.*, 2002). The first market introduction is expected for Japan and some Asian countries in the years between 2004 and 2005. This paper describes the technical properties and biological properties of pyridalyl.

CHEMICAL AND PHISICAL PROPERTIES

Code Number:	S-1812
ISO Name:	Pyridalyl
Chemical Name(IUPAC):	2,6-dichloro- 4-(3,3-dichloroallyloxy)phenyl 3-[5-(tri-fluoromethyl)-2-pyridyloxy] propyl ether
CAS RN:	179101-81-6

Structural formula:



Molecular Formula: $C_{18}H_{14}Cl_4F_3NO_3$

Molecular Weight:	491.1
Physical State at 20℃:	Liquid
Water solubility at 20℃:	0.15 ppb
Vapour tension at 20℃:	6.24×10^{-8} pa

FORMULATIONS

480 EC, 35 WP, 50 %EC, 10 % SC. Good compatibility with conventional crop protection products.

MAMMALIAN TOXICOLOGY

Acute oral LD_{50} , rat :	>5000mg/kg b.w. (males/females)
Acute dermal LD_{50}, rat:	>5000mg/kg b.w. (males/females)
Acute inhalation, LC_{50}, rat:	>2.01mg/liter
Eye irritation, rabbit:	Slight irritation
Skin irritation, rabbit:	No irritation
Skin sensitization, guinia pig:	Sensitizing

ECOTOXICOLOGY

Bobwhite quail, LC_{50} (in diet):	1133 mg/litre
Mallard duck, LC_{50} (in diet):	>5620 mg/litre
Rainbow trout, Acute toxicity LC_{50} (96hr):	0.50 mg/litre

MATERIALS AND METHODS

Table 1 lists the conditions for the four field studies described below in Tables 5-8.

Table 1. Details of field trials

Table	Insect	Spray volume (litres/ha)	No. of treatments	Evaluation based on	Evaluation days after last treatment
Table 5	*Heliothis virescens*	94	7	larvae/ 10 plants	3
	Spodoptera exigua	94	1	larvae/3 m of row	5
	Trichoplusia ni	94	4	larvae/3 m of row	2
Table 6	*Helicoverpa armigera*	40	1	larvae/m of row	4
	Spodoptera littoralis	450	1	larvae/ 10 plants	7
Table 7	*Helicoverpa zea*	138	6	% damaged fruits	6
	Spodoptera eridania	555-1110	8	% damaged fruits	12
	Tuta absoluta	500-1000	5	% damaged fruits	7
Table 8	*Plutella xylostella*	2000	1	larvae/ 10 plants	3
	Mamestra brasicae	2000	1	larvae/ 10 plants	3
	Thrips palmi	3000	1	insects/ 10 plants	7

BIOLOGICAL PROPERTIES

Laboratory studies

The insecticidal activity of pyridalyl toward various lepidopterous insects has been listed, as LC_{50} values (mg a.i./litre), in Table 2. The compound was also highly active against the insecticide-resistant strain of *P.xylostella* as well as susceptible strain (Table 3). Pyridalyl also showed good activity against thysanopterous insects at 100 mg a.i./litre (data are not shown).

Table 2. Insecticidal activity of pyridalyl against lepidopterous pests

Scientific name	Stage*1	Test method	DAT	LC_{50} (mg a.i./litre)
Cnaphalocrosis medinalis	L3	Foliar spray	5	1.55
Helicoverpa armigera	L3	Leaf dip	5	1.36
Helicoverpa zea	L2	Leaf dip	5	3.23
Heliothis virescens	L2	Leaf dip	5	4.29
Mamestra brassicae	L3	Foliar spray	5	1.98
Spodoptera exigua	L3	Leaf dip	5	0.93
Spodoptera litura	L3	Foliar spray	5	0.77
Pieris rapae	L2	Foliar spray	5	3.02
Plutella xylostella	L3	Leaf dip	3	4.48

*1 L2 and L3 means 2nd and 3rd instar larva, respectively.Table 3. Insecticidal activity of pyridalyl against insecticide resistant strain of *P.xylostella*

Table 3. Insecticidal activity of pyridalyl against insecticide resistant strain of *P.xylostella*

Insecticide	Class	LC_{50} (mg a.i./litre)	
		resistant strain	susceptible strain
pyridalyl		2.6	4.5
cyfluthrin	synthetic pyrethroid	> 500	3.7
pyrimifos methyl	organic phosphate	> 450	12.0
chlorfluazuron	benzoyl phenylurea	> 25	3.4

Pyridalyl showed little toxicity toward various beneficial arthropods at 100 mg a.i./litre (Table 4). It was also reported that pyridalyl had good selectivity to natural enemies in cotton (Tillman and Mulrooney, 2000).

Table 4. Beneficial arthropods not affected by pyridalyl at 100 mg a.i./litre

Scientific name	beneficials	Stage	Test method
Trichogramma japonicum	Egg parasitic wasp of lepidoptera	Adult	Foliar spray
Chrysoperla carnea	Predatory Chrysopidae	L2-3	Insect dip
Harmonia axyridis	Predatory Coleoptera	L2-3	Foliar spray
Orius sauteri	Predatory Hymenoptera	Adult/Nymph	Foliar spray
Phytoseiulus persimilis	Predatory Acarina	Adult	Foliar spray
Apis mellifera	Pollinator	Worker	Direct spray
Bombus terrestris	Pollinator	Worker	Direct spray

*1 L2 -3 means 2nd to 3rd instar larvae.

Field studies

Pyridalyl at 83-300 g a.i./ha provided excellent control of various lepidopterous pests and thrips on cotton and vegetables (Table 5 to 8). The compound also showed excellent efficacy against a population of *H.virescens* resistant to synthetic pyrethroids (Table 5). No phytotoxicity was observed in these field studies.

Table 5. Control (% Abbott) of lepidopterous pests on cotton in USA

Treatment	Rate (g a.i./ha)	*H.virescens* 1997	*S.exigua* 1998	*T. ni* 1998
pyridalyl	166	89	93	79
lambda-cyhalothrin	44	55	6	55
spinosad	70	75	49	73
untreated	-	(15)	(39)	(29)

untreated = number of live larvae

Table 6. Control (% Abbott) of *H.armigera* and *S.litoralis* on cotton

Treatment	Rate (g a.i./ha)	*H.armigera* Australia 1999	*S.litoralis* Turkey 1998
pyridalyl	100	89	88
pyridalyl	150	89	98
spinosad	96	68	-
thiodicarb	750	80	93
untreated	-	(4.3)	(266)

untreated= number of live larvae

Table 7. Control of lepidopterous pests on tomato

Treatment	Rate (g a.i./ha)	% damaged fruits		
		H zea USA 1999	*S.eridania* USA 1999	*T.absoluta* Brazil 2001
pyridalyl	83	3	-	-
pyridalyl	150	-	-	5
pyridalyl	166	3	3	-
lambda-cyhalothrin	33	5	-	-
spinosad	99	-	12	-
emamectin benzoate	15	6	5	-
lufenuron	50	-	-	7
untreated	-	24	24	32

Table 8. Control (%Abbott) of lepidopterous and thysanopterous pests on vegetables

Treatment	Rate (g a.i./ha)	*P.xylostella* on Cabbage Japan 1998	*M.brassicae* on Cabbage Japan 1998	*T.palmi* on Egg plant Japan 1997
pyridalyl	200	100	100	-
pyridalyl	300	-	-	100
emamectin benzoate	11	90	100	-
imidacloprid	150	-	-	97
untreated	-	(21)	(46)	(507)

untreated = number of live larvae

MODE OF ACTION

The symptoms in larvae of lepidopterous insects treated with pyridalyl are unique and different from any other existing insecticides. The insects treated with pyridalyl at lethal dose rates lost their vigour gradually and were killed in 2-3 hours. Moribund symptoms such as vomiting or convulsion were not observed in the treated larvae. Biochemical mechanism of insecticidal action is under investigation.

CONCLUSIONS

Pyridalyl shows very good efficacy for control of various lepidopterous and thysanopterous pests on cotton and vegetables without any phytotoxicity at practical dosages, which range from 83 to 300g a.i./ha. The biochemical mechanism of insecticidal action has not been identified at present, but it has different mode of action from any other existing insecticides because the compound shows good control of populations of *H.virescens* or *P.xylostella* resistant to various insecticides with unique insecticidal symptoms. Moreover, pyridalyl is less toxic to various beneficial arthropods. Consequently, pyridalyl will be an important material for lepidopterous and thysanopterous pest control under IPM or insecticide resistant management programmes.

ACKNOWLEDGEMENTS

The authors would like to thank all colleagues who contributed to the world-wide development of pyridalyl.

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BSN 2060: a novel compound for whitefly and spider mite control

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ABSTRACT

BSN 2060 (proposed common name: spiromesifen) is a novel insecticide/acaricide belonging to the new chemical class of spirocyclic phenyl-substituted tetronic acids. BSN 2060 is especially active against whiteflies, (*Bemisia* spp. and *Trialeurodes* spp.) after foliar application. Furthermore it is highly active against *Tetranychus spp.* as an acaricide in many cropping systems. Use rates of the non-systemic compound range from 100 to 150 g a.i./ha for whitefly and spider mite control in cotton, vegetables and ornamentals. The compound acts on mite and whitefly development, probably because it interferes with lipid biosynthesis. BSN 2060 is particularly active against juvenile stages. However, it also strongly affects fecundity of mite and whitefly adults in a dose dependent manner by transovariole effects. It shows excellent ovicidal effects in mites, whereas egg hatch in whiteflies was markedly reduced through transovariole effects upon pre-exposure of female adults. BSN 2060 was extremely effective against pyriproxyfen resistant whiteflies and represents a new valuable tool in whitefly resistance management when combined with neonicotinoid (chloronicotinyl) insecticides. No cross-resistance to any important insecticide and acaricide was found. Laboratory and field tests revealed that BSN 2060 is safe on beneficial organisms and suitable for integrated pest management (IPM) practices. It shows excellent residual activity, good plant compatibility and a favourable environmental profile.

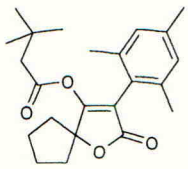
INTRODUCTION

Whiteflies, e.g. *Bemisia tabaci,* and tetranychid spider mite species such as *Tetranychus urticae* belong to the most serious sucking pests in many agricultural and horticultural cropping systems. They have developed a high degree of resistance against numerous chemical classes of insecticides and acaricides commercially available.

BSN 2060 is under development by Bayer CropScience and was discovered to be a potential whitefly insecticide and an excellent acaricide against tetranychid mite pests. It is the second member of a novel class of pest control agents invented by Bayer CropScience, the spirocyclic tetronic acid derivatives (Wachendorff et al., 2000). BSN 2060 will be registered world wide under the proposed brand name Oberon® 240SC as basic formulation. Market introduction is expected for South America, Europe and US between the years 2004 and 2006.

In this paper we present the physicochemical characteristics along with the toxicological and environmental behaviour of the active ingredient BSN 2060. Furthermore its biological performance in laboratory-, greenhouse and field trials is highlighted.

CHEMICAL AND PHYSICAL PROPERTIES

Common name (ISO):	spiromesifen
Chemical name (IUPAC):	3-(2,4,6-trimethylphenyl)-4-(3,3-dimethylbutyl-carbonyloxy)-5-spirocyclo-pentyl-3-dihydrofuranon-2
CAS number:	283594-90-1
Empirical formula:	$C_{23}H_{30}O_4$
Structural formula:	
Molecular weight:	370 g/mol
Colour/appearance:	colourless powder

HUMAN SAFETY

Acute oral LD_{50}, rat:	> 2500 mg/kg (males/females)
Acute dermal LD_{50} (24h), rat:	> 2000 mg/kg (males/females)
Acute inhalation LC_{50} (4h), rat:	> 4873 mg/m^3
Skin irritation (4h), rabbit:	non-irritant
Eye irritation (24h), rabbit:	non-irritant
Chronic toxicity (12 months), dogs:	NOAEL 50 ppm
Developmental toxicity:	no teratogenic potential (rats and rabbits), 2-generation rat study revealed no evidence of a primary reprotoxic potential
Genotoxicity:	no evidence of genotoxic or mutagenic potential

ENVIRONMENTAL SAFETY

Birds, acute toxicity LD50:	> 2000 mg a.i./kg
Earth worms, *Eisenia fetida*:	> 1000 mg a.i./kg of dry weight soil
Honeybee LD50 oral:	> 790 µg a.i./bee
contact:	> 200 µg a.i./bee
Ladybirds, *Coccinella septempunctata*:	not harmful
Predatory mites:	slightly to moderatly harmful

ENVIRONMENTAL FATE

Soil degradation, DT_{50}:	5 days
Soil mobility:	no leaching problems (Lysimeter study)
Vapour pressure:	7 x 10^{-6} Pa (20°C)
Water solubility:	0.13 mg/ml
Partition coefficient (logP):	4.55 (pH 2 and 7.5, 20°C, n-octanol/water)

FORMULATIONS

BSN 2060 will be formulated as a 24% suspension concentrate (SC 240) for Europe and USA. For professional nursery uses in the USA a SC 480 is under development. The formulations show good miscibility with conventional crop protection products.

BIOLOGICAL PROFILE

Whiteflies

Sweet-potato whiteflies, *B. tabaci,* including B-type *B. tabaci,* also known as *B. argentifolii,* and *Trialeurodes vaporariorum* are well controlled by BSN 2060. Juvenile stages, i.e. nymphs (1st to 3rd instar) are affected in the lower ppm range. Female adults of *B. tabaci* affected by foliar treatments of BSN 2060 showed a considerable decrease in fecundity. Typically, the number of eggs laid decreased dramatically and in a dose dependent manner. The decrease in fecundity was observed with concentrations as low as 8ppm. Higher concentrations, e.g. 200 and 40ppm, reduced the number of eggs laid by at least 90% compared to untreated control populations (Figure 1).

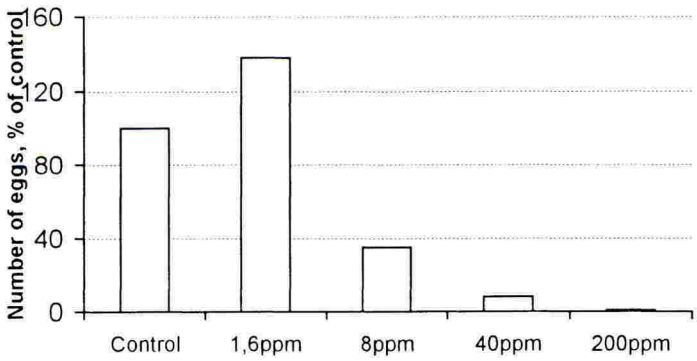


Figure 1. Effects of BSN 2060 on fecundity of *Bemisia tabaci* after transfer from foliar-treated to untreated cotton plants

Cross resistance studies

Resistance bioassays were done on cotton plants using 2nd instar whitefly nymphs employing protocols similar to those described by Elbert & Nauen (1996). BSN 2060 was more effective against 2nd instar larvae of *B. tabaci* than imidacloprid in a similar bioassay design (Elbert & Nauen, 1996). Those strains being resistant to organophosphates, carbamates, pyrethroids and endosulfan were definitely not cross-resistant to BSN 2060. Some of the Spanish (Almeria) Q-type strains tested, e.g. ESP-98 and ESP-00 are highly resistant to neonicotinoid insecticides in laboratory assays (Nauen et al., 2002), but hardly exhibit any cross-resistance to BSN 2060. Furthermore none of the pyriproxyfen-resistant *B. tabaci* strains showed any cross resistance to BSN 2060. These results indicate BSN 2060 as one of the most valuable tools in future whitefly control strategies.

Table 1. Log-dose probit-mortality data for BSN 2060 tested against nymphs of several strains of *Bemisia tabaci* in a leaf-dip bioassay (21d). Data were partially taken from a collaboration between Bayer CropScience and IACR Rothamsted, UK (Drs. G. Devine & I. Denholm)

Strain	LC_{50} [mg litre^{-1}]	Fiducial limits 95%	Slope	Known Resistance[1]
SUD-S	0.42	0.25-0.64	0.96	Susceptible
CAL-1	0.91	0.59-1.2	1.85	OP, CA, END, PYR
JAP-1	0.53	0.41-15	1.19	OP, CA, END, PYR
LMPA-2	0.54	0.18-0.98	1.35	OP, CA, END, PYR
ESP-98	0.34	0.083-0.74	0.94	Neonicotinoids
ESP-00	0.36	0.17-0.70	0.68	Neonicotinoids
PYRI-R	0.10	0.02-0.22	0.99	Pyriproxyfen
Koppert	1.03	0.50-1.8	1.29	Pyriproxyfen
PAK-9	2.00	1.1-3.1	1.25	OP, CA, END, PYR

[1] OP=Organophosphates, CA=Carbamates, END=Endosulfan, PYR=Pyrethroids

Field performance

Field trials in many parts of the world under different climatic conditions revealed excellent (residual) activity of BSN 2060 against different biotypes of *B. tabaci* (Figure 2).

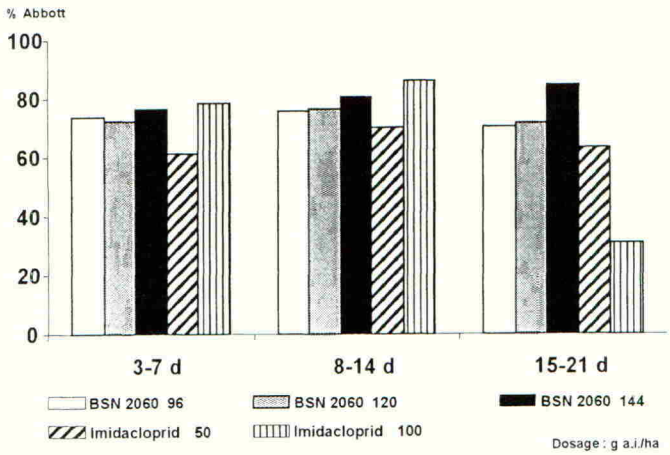


Figure 2. Efficacy of BSN 2060 SC 240 against Bemisia tabaci in cotton (combined data of several field trials in many countries)

Spider mites

BSN 2060 shows an excellent acaricidal potency and is active against all stages occuring during spider mite development; however, juvenile stages are often a little more susceptible than adults. French bean plants holding the desired developmental stage were foliarly treated with BSN 2060 using the same procedure as outlined in Nauen et al., 2000 (Figure 3).

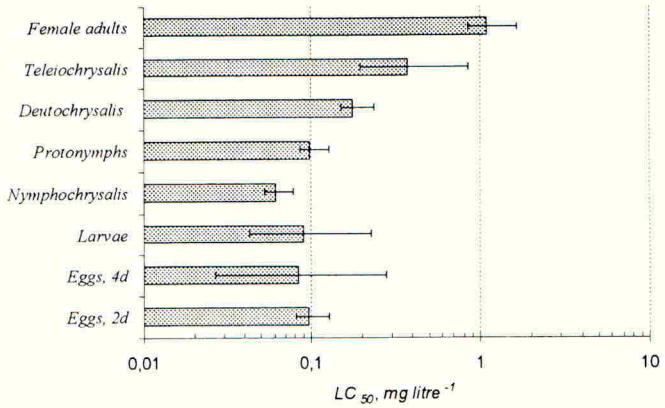


Figure 3. Efficacy (LC_{50}-values) of BSN 2060 against developmental stages of *Tetranychus urticae* (strain WI) 7d after spray application on French bean leaves

The potency of BSN 2060 against *T. urticae* hardly declined with progressive development. However, adults females and teleiochrysalis (quiescent stage just before adult-molt) were the least susceptible stages, i.e. LC_{50}-values were c. 5-10-times higher than against larvae.

Cross resistance studies

No cross-resistance to conventional acaricides such as pyridaben, fenpyroximate (and all other METI´s), abamectin, hexythiazox, clofentezine, dicofol and organophosphates was detected in several strains of *T. urticae* tested throughout this study. Due to the structural similarity between spirodiclofen and BSN 2060 it can be assumed that conclusions drawn from spirodiclofen resistance assessment (Nauen *et al.*, 2000) are to a greater or lesser extent also applicable for BSN 2060.

Table 2. Resistance factors for different acaricides based on LC_{50}-values in several strains of *Tetranychus urticae*. Strains were described in Nauen et al, 2000 and Stumpf & Nauen, 2001

Acaricide	WI	NL-00	AKITA	UK-99	AU
Abamectin	1	54	3	-	2
Pyridaben	1	22	2000	860	13
Fenpyroximate	1	-	1400	74	5
Hexythiazox	1	-	4	-	1100
Clofentezine	1	-	4	-	>770
Spiromesifen	1	4	1	1	3

Field performance

BSN 2060 showed excellent activity against *T. urticae* in many field trials worldwide (an example is given in Figure 4).

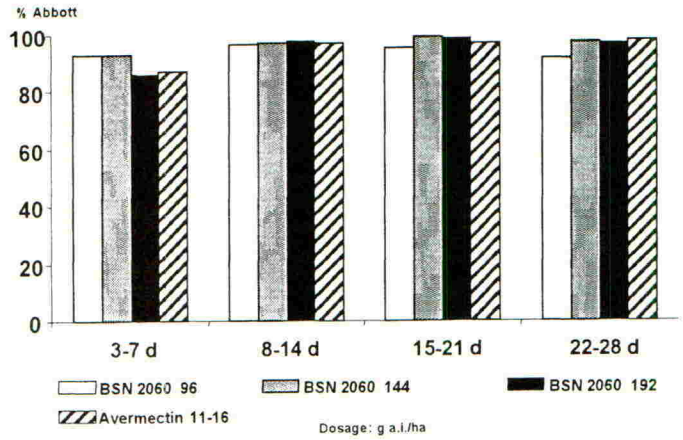


Figure 4. Efficacy of BSN 2060 SC 240 against Tetranychus urticae in eggplants (several field trials in different countries combined)

CONCLUSIONS

BSN 2060 is a new IPM-suitable insecticide/acaricide which exhibits an excellent activity against whiteflies and tetranychid mite pests combined with a very good plant compatibility. It belongs to the new group of tetronic acid derivatives with a new mode of action and no cross-resistance to any other commercially available acaricide. BSN 2060 will be an excellent resistance management tool in agronomic cropping systems and professional nurseries.

ACKNOWLEDGEMENTS

Thanks to all colleagues who contributed to the worldwide development of BSN 2060.

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Field and laboratory studies on the effects of a neem-based plant extract on the feeding activity of the large pine weevil, *Hylobius abietis*

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ABSTRACT

Field and laboratory studies were carried out from September to October 2001 to assess the effects of a neem-based plant extract on the feeding activity of the large pine weevil *Hylobius abietis* in a commercial sitka spruce plantation. Field trials on Forestry Commission land near Aberfoyle assayed the effects of extract concentration, dosage, and treatment area on feeding activity. Laboratory experiments assayed the effects of the extract on feeding activity in choice and no-choice situations. The results of both field and laboratory studies indicated that the extract had a significant deterrent effect on weevil feeding activity. In the field, untreated seedling trees suffered up to 100% feeding damage while many of the trees that were treated with the extract remained free of pest damage throughout the sampling period. Preliminary conclusions indicate that the neem extract deters this pest species from feeding. The results are discussed within the context of pest control in commercial forestry in Scotland.

INTRODUCTION

The large pine weevil (*Hylobius abietis*) is the single most important pest of young conifer transplants in commercial forestry in the UK. Adult female weevils are attracted to felling sites by the volatile chemicals (mainly pinene and ethanol) that are released from the stumps of felled trees. These beetles then lay eggs under the bark of stumps and feed on the lower section of the main stem of restocked seedling trees. When the feeding damage on newly replanted seedlings is severe, the trees will die. Larvae of the beetle feed under the bark of stumps and roots of felled trees and emerge as adults following pupation after a period of 1 – 2 years. These emerging adults then also feed on newly restocked seedlings. The level of damage caused by the beetle varies with location, with conifer species, and with tree age. On restocking sites that have large beetle populations 100% of unprotected, newly planted trees have been killed. The UK national average for losses of unprotected trees is estimated to be *ca.* 50%. Typically, newly planted seedling conifers may be susceptible to the beetle for 1 – 4 years after planting. As a result, *H. abietis* is the only UK forestry pest against which prophylactic treatments with insecticide are routine.

At present, beetle damage is minimised by treating seedling trees with insecticide. In the UK all seedling trees destined for a restock site are treated the pyrethroid insecticide permethrin by dipping or spraying. These treatments usually provide protection during the first growing season for spring planted nursery stock. Further protection of trees in subsequent years can be achieved by spraying insecticides with a knapsack sprayer. These treatments protect the trees from beetle damage they do not control the pest population. In other words, the current strategy for minimising pest damage is based upon plant protection rather than pest control. Protecting trees from attack has no known effect on the overall pest population at a particular location.

In 2003 approval for treating seedling conifers with permethrin in the UK will be will be phased out. As a result, there are now a number of initiatives associated with developing alternatives methods of seedling protection. These initiatives include the use of other insecticidal products, the use of plant extracts with antifeedant properties (Klepzig & Schlyter, 1999), the use of nematodes for pest control (Brixey, 1997), and the development of more complex integrated forestry management programmes that may enable more accurate targeting and/or the elimination of the need for chemical control products (Heritage & Moore, 2001). Alternative insecticidal products that are currently under evaluation include the synthetic pyrethroid insecticides alpha-cypermethrin and lambda-cyhalothrin and a number of natural compounds derived from various plant species. In the research described in this paper we report the first results of experiments that were undertaken to assay the effectiveness of one plant-based product for minimising pine weevil damage in forestry. The product we assayed was a neem-based extract derived from commercial plantation mahogany production in India.

MATERIALS AND METHODS

The neem-based extract was provided as a semi-solid formulation under the tradename Bugban. At present, this formulation is not registered for use in forestry within the UK. The material safety data sheet for this product indicates that it comprises tetranortriterpenoids and the molecular weight and empirical formula for the principal active ingredient were listed as those for the compound azadirachtin, the principal active ingredient of neem extracts. To confirm the identity of the principal chemical compounds within the extract biochemical analyses were carried out.

Biochemical analysis of the plant extract

To identify the compounds present in the plant extract a High Performance Liquid Chromatography (HPLC) assay was carried out using a reverse phase C18 column. In this assay 1g of the Bugban was dissolved in 10ml of methanol, filtered and then 50 µl loaded into the HPLC machine using the gradient elution method where the composition of the solvent varied from 50% - 100%. The retention times and peaks of compounds detected in the assay were compared to a neem oil standard (courtesy of Shri Disha Biotech Ltd. Hyderabad, India) that was also run through the HPLC assay. Detection was by UV absorbance at 217 nm.

Field experiments

All field trials were carried out on Forestry Commission land near Aberfoyle, Scotland. In total, 96 seedling sitka spruce were planted in a grid with 2 m spacing in a randomised block design. Eight replicate trees were used per treatment. The seedling trees were approximately 2

years old. The field experiments assayed the effects of extract concentration, dose, and treatment area on beetle feeding activity. All treatments were applied by brush around the root collar of the seedling trees. After treatment application the seedlings were left to dry for 1 h before planting. Control trees were untreated. The concentration of the plant extract was changed by dilution with vegetable oil. In the first field experiment 48 trees were used. These trees were treated with 5 ml of the plant extract from 0 - 15 cm above the root collar. The concentrations used in the first experiment comprised 100%, 80%, 60%, 40%, 20%, and 0%, i.e. control. In the second field experiment 24 trees were used to assay the effects of extract treatment area. The trees were treated with 5 ml or 10 ml of the extract for 0 - 15 cm or 0 - 30 cm above the root collar. In this second experiment the extract was used neat, i.e. a concentration of 100%. The third field experiment assayed the effects of treatment dosage. Trees were treated with 5 ml or 10 ml of neat plant extract for a distance of 0 - 15 cm above the root collar. Feeding damage to the trees was then assessed weekly from September to November 2001 using a percentage scoring system. Damage was assessed at 0 – 15 cm above the root collar and at 15 – 30 cm above the root collar. A score of 0% indicated no feeding damage and a score 100% indicated that all of the bark had been removed within the assessment zone. The data were analysed using two-way analysis of variance following arcsine-squareroot transformation.

Laboratory experiments

Laboratory experiments comprised assaying the effects of the plant extract on beetle feeding activity by confining beetles in petri dishes with sitka spruce twigs. Two beetles were confined with two twigs that were either both untreated (control), both treated (no-choice), or with one twig treated (choice). Eight replicate petri dishes were used per treatment. The laboratory experiments assayed the effects of treatment concentration, dose rate, and application pattern. Here we report the results of the experiments that assayed the effects of the neat plant extract on beetle feeding activity. In this experiment 6 cm long sitka spruce twigs with a 1 cm diameter were used. These twigs were treated with 2 ml of neat extract using a brush. Needles were removed from the twigs and the ends dipped in melted wax. The twigs were left to dry for 1 hr after treatment application and were then placed on a wet filter paper in the petri dish. Two field collected weevils were placed in the petri dish with the twigs. The gender of the weevils was not determined. The petri dishes were stored at room temperature. The percentage bark removed from the twigs was assayed weekly for two months, as described above. The data were analysed using two-way analysis of variance following arcsine-squareroot transformation

RESULTS

The main compounds detected in HPLC assay of the plant extract were identified as azadirachtin, nimbin and salanin. The size of peaks and retention times for these compounds were identical to those recorded with a neem oil standard. The analysis therefore identified the plant extract as a crude neem oil formulation unadulterated with additives.

Field experiments

Figure 1 shows the mean percentage bark that was removed from around the root collar of the seedling trees for different concentrations of the plant extract. The data show that there was a

highly significant treatment effect ($F = 108.6$, $P < 0.001$). Time did not have a significant effect upon the data ($P = 0.91$) and there was not a significant interaction between treatment and time ($P > 0.05$). The results therefore indicate that the deterrent effects of the extract are immediate and persistent. All of the untreated trees and those treated with an extract concentration of 20% lost approximately 70% of their bark during the sampling period. By contrast trees treated with extract concentrations of 60 – 100% lost less than 20% of their bark.

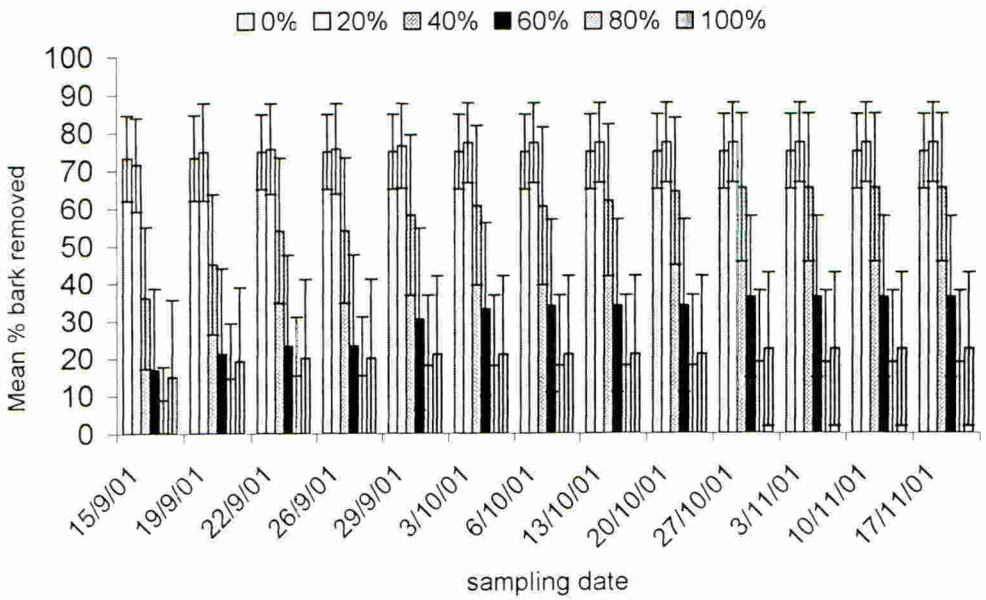

Figure 1. Mean percentage bark removed from 0 - 15 cm above the root collar (+/- 95% confidence limits) for different extract concentrations.

Figure 2 shows the mean percentage bark removed over 0 - 15 cm or 15 – 30 cm with neat (100%) extract. The results show that the extract had a highly significant treatment effect ($F = 477.7$, $P < 0.001$). Time did not have an impact upon the treatment effect ($P = 1$). Untreated trees suffered losses of approximately 60% while treated trees suffered losses of approximately 10%. The figure shows that there was no feeding activity in the 15 – 30 cm zone. Since beetles feed around the root collar this was not unexpected. Figure 3 shows the mean percentage bark removed over 0 – 15 cm with neat extract applied as either 5 ml or 10 ml. As before the extract has a highly significant effect upon beetle feeding activity ($F = 363.4$, $P < 0.001$). Time had no impact on this repellent effect. The results show that there were no differences between using 5 or 10 ml of extract. Overall, untreated trees suffered losses of approximately 60% while treated trees suffered average losses of < 10%.

Laboratory experiments

Figure 4 shows the mean percentage bark removed from twigs in the laboratory experiments. Control twigs were always untreated, i.e. both twigs labelled 'one' and 'two'. In the no-choice experiment both twigs were treated. In the choice experiment only the twig labelled 'one' was treated with the neat extract. The results show that the extract significantly reduced feeding on

treated twigs ($F = 152.5$, $P < 0.001$). The analysis of variance also indicated that there

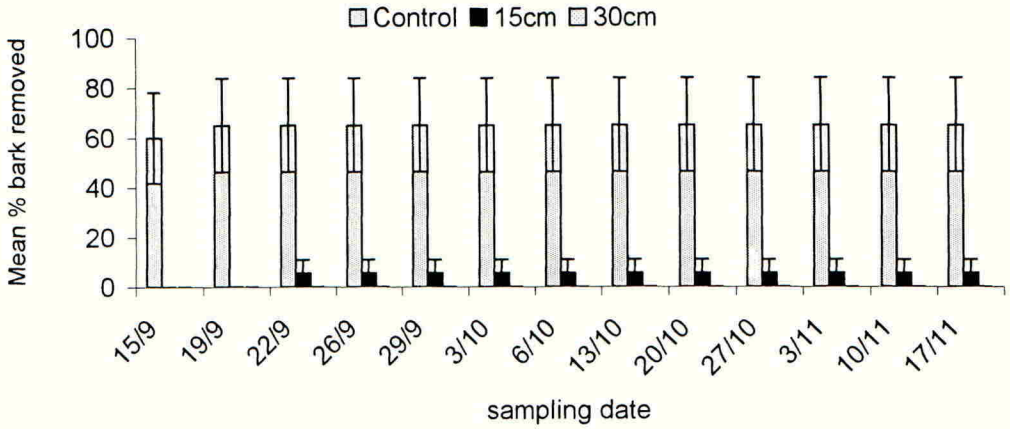

Figure 2. Mean percentage bark removed around the root collar (+/- 95% confidence limits) for treatment zones (0 – 15 cm or 15 – 30 cm) with neat extract.

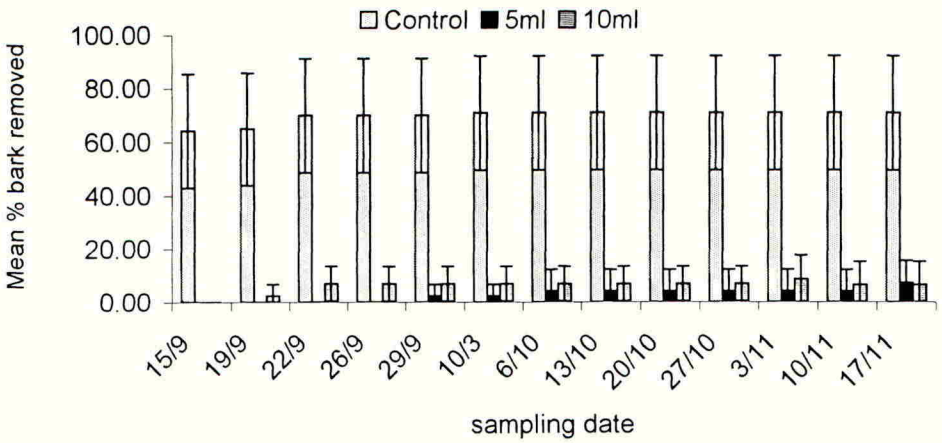

Figure 3. Mean percentage bark removed from 0 – 15 cm above the root collar (+/- 95% confidence limits) for treatment volumes (5 ml or 10 ml) with neat extract

were statistically significant time and interaction effects. The amount of bark removed from control twigs increased as the experiment progressed and after 6 weeks approximately 50 – 60% of bark had been removed. In the no-choice experiment the amount of bark removed from both twigs was approximately 20% by the end of the experiment. This amount was significantly lower than the control. None of the beetles that that fed on treated bark died. In the choice situation the feeding activity on the untreated twig was significantly higher than that on the treated twig.

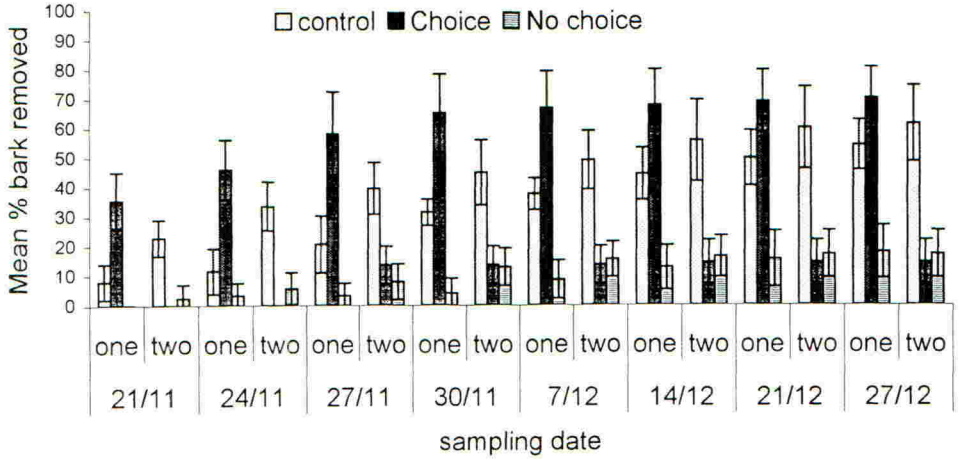

Figure 4. Mean percentage bark removed from twigs (+/- 95% confidence limits) in petri dishes treated with 2 ml of neat plant extract.

DISCUSSION

The field and laboratory data presented in this paper clearly indicate that the plant extract deterred feeding by *H. abietis* on sitka spruce. Almost all of the control trees died during the course of the field experiment as a result of feeding activity by the weevil while a significant number of trees treated with the extract remained free of pest damage. As a consequence, experiments are currently (2002) underway to compare the efficacy of the plant extract with commercialised neem formulations. In the data presented, the extract was effective for the duration of the sampling period (2 – 3 months), however we also need to evaluate the exact time period over which the extract repels beetles. Further data are also required on the most effective dose and application method for the extract. Overall, it would appear that the extract might have a role to play in protecting seedling trees from attack by the large pine weevil. The use of a neem extract would fit with the current government's strategy of using environmentally friendly products for pest control, however whether it would be economic to develop the extract assayed here for use in forestry pest management remains to be seen.

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Clothianidin: a novel broad-spectrum neonicotinoid insecticide

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ABSTRACT

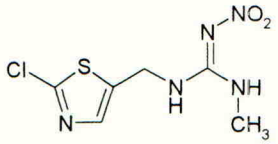
Clothianidin (TI-435) is a novel neonicotinoid insecticide, acting as an agonist of nicotinic acetylcholine receptor (nAChR). This compound has minimal adverse effects against vertebrates. The potent agonistic action of clothianidin was observed only on insect nAChR, but not on vertebrate ones, indicating that the compound has selective toxicity for insects over vertebrates. Laboratory studies have demonstrated that clothianidin is highly active against not only hemipterous insects but also coleopterous, thysanopterous, dipterous and some lepidopterous pests. Since this compound possesses excellent root systemic properties, it can be used by various application methods. In field trials, clothianidin exhibited excellent control of insect pests by foliar application, paddy water application, soil application and seed treatment. Because of its broad spectrum of insecticidal activity, good systemic properties and low mammalian toxicity, clothianidin is a compound that is considered to be compatible with integrated pest management strategies.

INTRODUCTION

Clothianidin is a neonicotinoid insecticide that was discovered by Takeda Chemical Industries Ltd. and is under worldwide joint development with Bayer CropScience. Belonging to the same chemical class of insecticides, Takeda has already developed and marketed nitenpyram since 1995. As a result of this continuing study on neonicotinoids, some nitroguanidin derivatives with thiazol-5-ylmethyl moiety were found to show increased activity against certain lepidopterous pests. After optimization of these derivatives, clothianidin was selected as the most promising compound for further development.

CHEMICAL AND PHYSICAL PROPERTIES

Structural formula



ISO common name:		clothianidin
Code number		TI-435
Chemical name		(*E*) -1- (2-chloro-1,3-thiazol-5-ylmethyl) -3-methyl-2-nitroguanidine
CAS Registry No.		210880-92-5
Molecular formula:		$C_6H_8ClN_5O_2S$
Molecular weight		249.7 g/mol
Appearance		White crystalline powder
Odour		Odourless
Melting point		176.8℃
Vapour pressure		1.3×10^{-10}Pa (25℃), 3.8×10^{-7}Pa (20℃)
solubility (g/l):	water	0.327 (20℃)
	acetone	15.2 (25℃)
	methanol	6.26 (25℃)
	ethyl acetate	2.03 (25℃)
	Xylene	0.013 (25℃)
Partition coefficient (n-octanol/water)		0.7 (log P_{ow}) (25℃)

MAMMALIAN TOXICITY

Acute oral LD_{50}	Rat (male; female)	>5000 mg/kg ; >5000 mg/kg
Acute dermal LD_{50}	Rat (male; female)	>2000 mg/kg ; >2000 mg/kg
Acute inhalation LC_{50}	Rat (male; female)	>6.1 mg/L ; >6.1 mg/L
Eye irritation	Rabbit	Non-irritant
Skin irritation	Rabbit	Non-irritant
Skin sensitization	Guinea pig	Non-sensitizer

EFFECTS ON NON-TARGET ORGANISMS

Bobwhite quail LD_{50} (oral)	>2000 mg/kg
Bobwhite quail LC_{50} (dietary)	>5200 ppm
Mallard duck LC_{50} (dietary)	>5200 ppm
Rainbow trout LC_{50} (96 hr)	>100 mg/L
Bluegill LC_{50} (96 hr)	>120 mg/L
Daphnia EC_{50} (48 hr)	>120 mg/L
Green algae E_bC_{50} (72 hr)	>270 mg/L
Earthworms LC_{50} (14 d)	13.21 mg/kg dry soil

INSECTICIDAL PROPERTIES

Mode of action

The agonist actions of clothianidin on chicken neuronal α4β2 nicotinic acetylcholine receptor (nAChR) and *Drosophila* SAD/chicken β2 hybrid nAChR were investigated by voltage clamp electrophysiology as described earlier by Matsuda *et al.* (Matsuda et al.,1998). The recombinant chicken α4β2 and hybrid SADβ2 nAChRs were expressed in *Xenopus oocytes* by injecting 1:1 mixture of the α (α4 or SAD) and non-α (β2) cDNA solution into nucleus. The compounds were bath-applied by a gravity fed system. Clothianidin did not activate the α4β2 receptor. In contrast, the compound acted as a potent agonist on the *Drosophila* SAD/chicken β2 hybrid receptor with the maximum amplitudes of responses of the SAD β2 receptor to clothianidin being significantly greater than those to acetylcholine (ACh) at saturating concentrations (Matsuda. *et al.*, 2001) (Figure 1). These agonist profiles of clothianidin are likely to be related to its selective toxicity for insects over vertebrates.

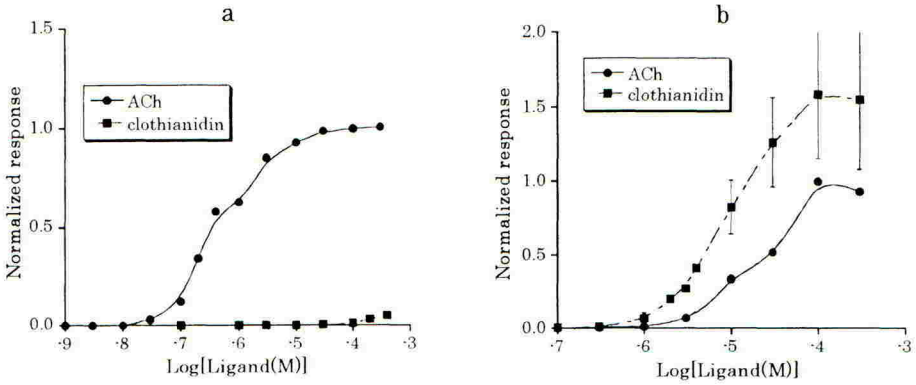


Figure 1 Dose-response relationship for clothianidin and acetylcholine obtained for recombinant α4β2 (a) and SADβ2 (b) receptors. Each data point represents the mean of 3 – 6 experiments ; vertical lines show s.e. about the mean.

Laboratory tests

Insecticidal spectrum

The LC_{50} values of clothianidin were very low not only for hemiptera but also for coleoptera, thysanoptera, diptera and some lepidoptera (Table 1). The insecticidal activities of clothianidin against most of the insect pests were demonstrated to be higher than those of fenitrothion.

Translocation and systemic action

Translaminar activity

The translaminar activity of clothianidin against cotton aphid, *Aphis gossypii* was examined by spraying the upper surface of a cucumber leaf with clothianidin solution after placing the adult females onto the undersurface of the leaf. Clothianidin exhibited high translaminar activity with an EC_{50} value of 0.35 mg/L.

Translocation from leaves to leaves

Cucumbers of seven-leaf stage were sprayed with the clothianidin solution, with the first and newly developing leaves being covered with polyethylene bags to keep them free from spray deposits. At 2 and 8 days after treatment, adult cotton aphid females were placed onto the untreated leaves, and the numbers of aphids were counted 6 days after the inoculation. Clothianidin significantly reduced the number of aphids not only on the upper untreated leaves but on the lower untreated leaf (Table.2).

Translocation from roots to leaves

The uptake of clothianidin from the root of a cucumber of two-leaf stage was evaluated after application by root dipping. Adult females of cotton aphid were inoculated to leaves one day after treatment and the number of aphids was counted 6 days after the inoculation. Clothianidin exhibited excellent root systemic activity with an EC_{50} value of 0.0015 mg/L. The outstanding root systemic property of clothianidin is considered to be preferable for soil and seed treatments.

Table 1. Insecticidal activity of clothianidin

Species	Stage [a]	Methods [b]	LC_{50}(mg a.i./L)		
			Clothianidin	Fenitrothion	Etofenprox
Hemiptera					
Nilaparvata lugens	N3	LS	0.015	41.1	13
Laodelphax striatellus	N3	LS	0.025	4.14	5.6
Sogatella furcifera	N3	LS	0.015	6.59	2.2
Nephotettix cincticeps	N3	LS	0.0006	4.47	2
Aphis gossypii	A	LS	0.011	0.87	0.81
Myzus persicae	A	LS	0.21	>20	0.54
Bemisia argentifolii	N1	LS	0.3	>100	4.7
Plautia stali	A	FS and IS	4.8	-	-
Coleoptera					
Henosepilachna vigintioctopunctata	L2	FD	0.051	1-2	1.8
Diabrotica undecimpunctata	L1	SI	0.16	-	-
Thysanoptera					
Thrips palmi	L1	LS	5.4	301.4	-
Frankliniella occidentalis	L1	LS	6.1	109	-
Lepidoptera					
Chilo suppressalis	L3	LS	0.28	6.59	-
Spodoptera litura	L3	LS	2.86	7.7	-
Plutella xylostella	L2	LD	59	3.1	3.2
Carposina niponensis	E	ED	0.24	1.24	-
Diptera					
Liryomyza trifforii	L1	LS	1.16	>100	>100

[a] E: eggs, N: nymph, L: larva, A: adult, the numeral indicates the instar number.

[b] LS: leaf spray, FS: fruit spray, IS: insect spray, LD: leaf dipping, FD: fruit dipping, ED: egg dipping, SI: soil incorpol

Table 2. Insecticidal activity against cotton aphid (Aphis gossypii) of clothianidin translocated from the treated leaves to untreated leaves.

Compound	Concentration (mg/L)	Leaf [a] position	% Control	
			2DAT	8DAT
Clothianidin	50	1st	99	- [b]
		8th	100	-
		9th	-	98
		10th	-	91
	100	1st	99	-
		8th	99	-
		9th	-	96
		10th	-	92

[a] The leaf position was counted from the basal true leaf.

[b] Not tested

Field performance

Clothianidin is expected to be used with various application methods because of its excellent systemic action. Aphids and whiteflies are very important insect pests, and rice planthoppers are one of the major pests of rice. Field trials against these homopterous insect pests provide good examples for demonstrating the performance of clothianidin with various application methods.

Foliar application

A solution of water soluble granules of clothianidin sprayed onto eggplants at the rate of 50-100 mg a.i./L provided good control of aphids (*Aphis gossypii*, *Myzus persicae*) for more than 3 weeks and showed no phytotoxicity.

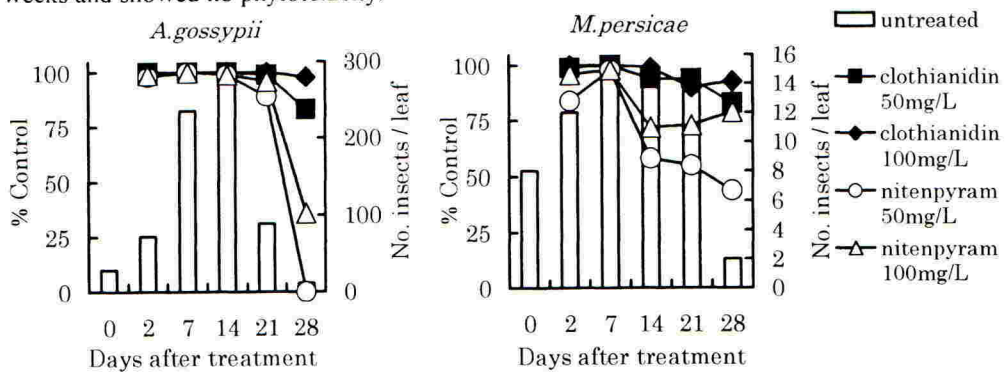


Figure 2. Control of aphids on eggplants by foliar spray of the chemical solutions.Each line with symbols indicates the control ratio of respective chemical application. Open bars indicate the number of insects per leaf in the untreated plot.

Paddy water application

Granule formulations of each chemical were applied to the paddy water by top dressing. Clothianidin showed no phytotoxicity and long lasting control of brown rice planthopper (*Nilaparvata lugens*) and green rice leafhopper (*Nephotettix cincticeps*) at the rate of 50-100 g a.i./ha.

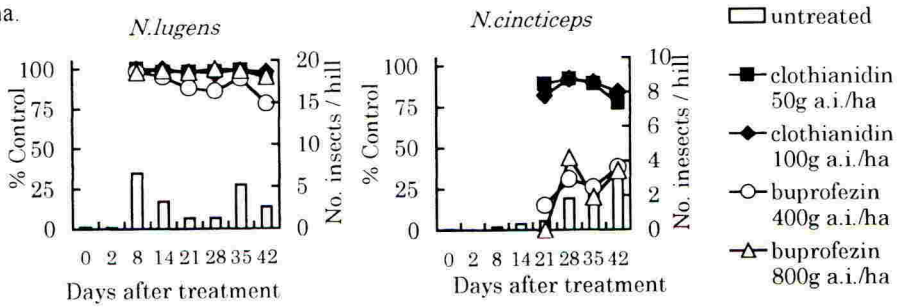


Figure 3 Control of brown rice planthopper and green rice leafhopper by paddy water application. Each line with symbols indicates the control ratio of respective chemical application. Open bars indicate the number of insects per hill in the untreated plot.

Soil application

Seedling box application

Granule formulations of each chemical were applied to rice seedlings in a seedling box just before transplanting. Good control of brown planthopper (*Nilaparvata lugens*) up to heading stage of rice was obtained by the application of clothianidin at the rate of 150 g a.i./ha. No phytotoxicity was observed.

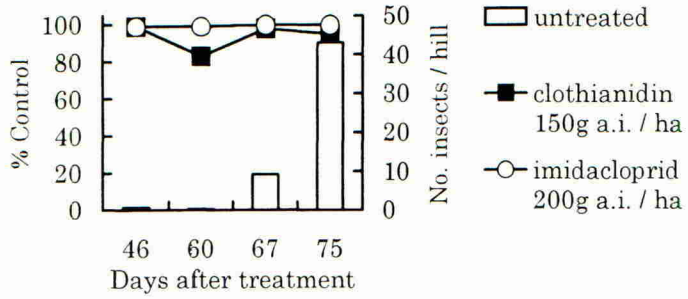


Figure 4 Control of brown planthopper (*Nilaparvata lugens*) by seedling box application. Each line with symbols indicates the control ratio of respective chemical application. Open bars indicate the number of insects per hill in the untreated plot.

Planting hole application and application to plant bases

Granular formulations of each chemical were applied to the planting hole prior to transplanting (PHA) or to the plant foot just after transplanting (PFA) of eggplant. Clothianidin by both application methods showed no phytotoxicity and good control of aphids (*Aphis gossypii*, *Myzus persicae*) for more than 56 days at the rate of 5 mg a.i./plant.

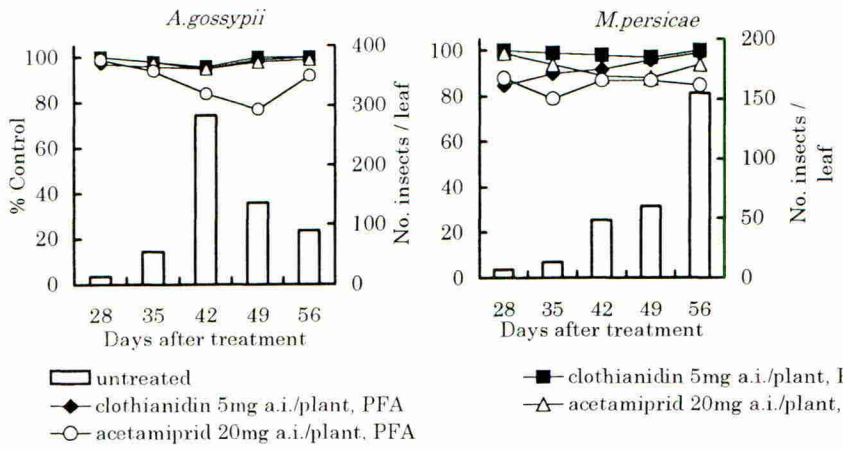


Figure 5. Control of the aphids on eggplants by planting hole application (PHA) and plant foot application (PFA). Each line with symbols indicates the control ratio of respective chemical application. Open bars indicate the number of insects per leaf in the untreated plot.

Nursery-pot-soil incorporation and soil drench of chemical solution

The control efficacy of clothianidin against greenhouse whitefly (*Trialeurodes vaporariorum*) on tomato were compared among the following application methods, which were :

1) tomato of 2nd leaf stage was transplanted to the 300ml pot with the soil incorporated with granule formulation of clothianidin (SI),
2) chemical solution of clothianidin was applied to the foot of potted tomato plant 3 days before transplanting (SD),
3) granule formulation of clothianidin was applied to the planting hole prior to transplanting of tomato (PHA).

With all of the application methods, Clothianidin effectively suppressed the population density of greenhouse whitefly for more than 56 days at the rate of 2.5 mg a.i./plant. No phytotoxicity was observed in any of the applications.

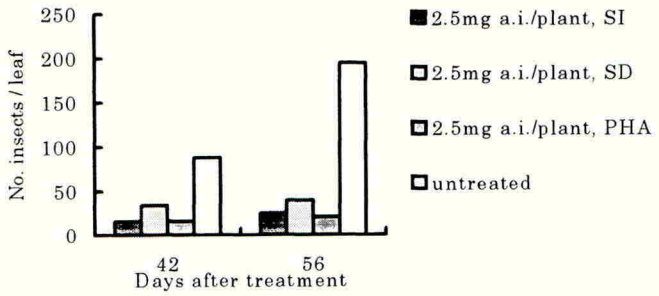


Figure 6 Control of greenhouse whitefly (*Trialeurodes vaporariorum*) on tomato by various soil applications of clothianidin. SI : nursery-pot-soil incorporation; SD : soil drench of chemical solution; PHA : planting hole application. Each bar indicates the number of insects / leaf in respective plot

Seed treatment

Corn seeds were treated with clothianidin solution at the rate of 200-400 g a.i./100 kg seeds. Clothianidin showed excellent control of wheat aphid (*Rhopalosiphum padi*) and no phytotoxicity.

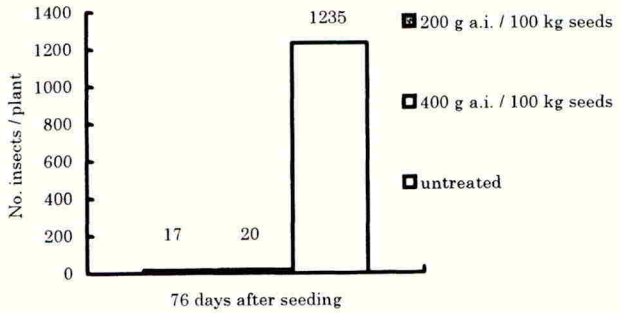


Figure 7 Control of wheat aphid (*Rhopalosiphum padi*) on corn by seed treatment. Each bar indicates the number of insects per plant in the respective plot.

Further details about the efficacy profile of clothianidin through seed treatment are given in Meredith *et al.* (2002) and Schwarz *et al.* (2002).

CONCLUSIONS

The properties of clothianidin are summarized as follows:

1. Highly active against a broad-spectrum of insect pests.
2. Very low toxicity against mammals, birds, fishes and crustaceans.
3. The difference of clothianidin sensitivity of the vertebrate and *Drosophila*-vertebrate hybrid nicotinic acetylcholine receptors seems to suggest selectivity of the compound to insects over vertebrates.
4. Highly systemic in plants and highly safe to crops.
5. Foliar spray, paddy water application, various methods of soil application and seed treatments are available.
6. Long-lasting control of insect pests can be achieved by the application of clothianidin at low rates.

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Meredith R H, Heatherington P J, Morris D B (2002) Clothianidin - a new chloronicotinyl seed treatment for use on sugar beet and cereals : Field trial experiences from Northern Europe. *Proceedings of the British Crop Protection Conference – Pests &Diseases*, 2002.. Brighton. UK.

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Control of corn rootworms (*Diabrotica spp.*) and of secondary pests of corn (*Zea mays*) using seed treatments of clothianidin

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ABSTRACT

Clothianidin, a new neonicotinoid insecticide for seed treatment, has been extensively tested against major insect pests of corn, canola, and other crops. The compound is highly root systemic and enters the transpiration stream through the roots of newly germinating seedlings and developed plants. Pests become intoxicated mainly through ingestion of protected plant tissues and stop feeding immediately. In tests for control of Western, Northern, Southern, and Mexican corn rootworm (*Diabrotica* spp.), clothianidin demonstrates a consistent reduction of root damage that is comparable to currently applied organophosphate, pyrethroid, and fipronil-based soil insecticides. In 112 trials conducted from 1997 through 2001 across the North American corn belt, clothianidin at 1.25 mg a.i./kernel gave an average Iowa root damage rating (Iowa 1-6 scale, IRDR) of 2.84, similar to chlorpyrifos (2.78), soil-applied tefluthrin (2.60), and fipronil (2.86). Untreated checks averaged an IRDR of 4.35. Only 26% of clothianidin-protected roots gave an IRDR greater than the economic threshold of 3.5, a good measure of treatment consistency, and similar to established organophosphate (18%) and fipronil (18%) corn soil insecticides.

Clothianidin also shows excellent control of most important secondary pests of corn in North America when tested as a seed treatment at rates of 0.125 to 0.5 mg a.i./kernel. The compound has good activity against wireworm (*Melanotus spp.*), seed corn maggot (*Hylemyia platura*, etc.), flea beetle (*Chaetocnema pulicaria*), chinch bug (*Blissus leucopterus*), white grub (*Lachnosterna implicita*), Southern green stink bug (*Nezara viridula*), and grape colaspis (*Colaspis brunnen*). The compound shows good activity for black cutworm (*Agrotis ypsilon*) at rates of 0.25 mg a.i./kernel. Control of corn rootworm and secondary pests resulted in a significant increase in yield up to 17.6 percent on the average compared to control plots. The high relative performance of this seed treatment compared to existing soil insecticides will be presented. The excellent fit for this compound in IPM and IRM strategies is discussed.

INTRODUCTION

Corn rootworms (*Diabrotica spp.*) are the major soil inhabiting corn pests in North America. *Diabrotica* larvae feed on primary and secondary roots. Plants with severe root feeding damage have poor stability that can result in lodging and reduced harvest efficiency - and

reduced yield. Feeding damage on the roots will also have an impact on root and plant growth and, therefore, on the yield, especially if the climatic conditions become unfavourably dry. According to 1999 Doane Market Research, there are about 12.3 million hectares of corn treated every year with insecticides for corn rootworm and cutworm control.

Control of corn rootworm has been managed conventionally through crop rotation or application of granular/ liquid soil insecticides. Clothianidin has been extensively tested for control of corn rootworm and secondary pests. The compound was applied as a film coating to the seeds and was compared directly with commercial soil insecticides.

Clothianidin is under joint development of Bayer CropScience for seed treatments and Takeda Chemical Industries, Ltd. for soil and foliar applications. The properties of this compound are presented by Ohkawara *et al.* (2002). Its registration as seed treatment is expected in North-America and Europe as early as 2003 under the brand name Poncho®.

MATERIALS and METHODS

Corn (*Zea mays*) seed was routinely coated with a 600 FS formulation of clothianidin using professional seed treatment equipment at a rate of 1.25 mg a.i./ kernel for control of corn rootworm or 0.125 to 0.5 mg a.i./ kernel as indicated for control of secondary pests. All seed was protected against soil-borne pathogens by standard seed treatment fungicides. For comparison, commercial soil insecticides were applied as granular or liquid formulations at their recommended rates as in-furrow or T-band applications.

All corn research was completed by university and industry researchers at key locations throughout the corn growing areas. Consequently, specific materials and methods varied somewhat between locations/researchers, but all conformed to the following standard procedures. The evaluation of the root damage was carried out approximately 8 to 10 weeks after sowing. Representative corn plants were randomly chosen from each replicated plot, dug out and the roots were thoroughly washed with water to remove soil and debris. Larval feeding damage to the root mass was then determined according to the Iowa Root Damage Rating (IRDR) scheme (Hill & Peters, 1971).

The efficacy of the compounds/ products against secondary pests was evaluated either by counting the plant stand, assessing the plant height or uniformity (grubs, wireworms, cutworms, grape colaspis, seed corn maggots), by counting the number of pests per plant (bugs, aphids) or similar measure as indicated.

Corn yield was determined by using machine harvesters on the middle two rows of four-row plots and recording the weight of grain harvested from each plot.

RESULTS

Efficacy of clothianidin seed treatment against corn root worms

"Corn rootworm" is the general term comprising different species of *Diabrotica*. Clothianidin is very active against the most abundant western (*D. virgifera virgifera*) and northern (*D.*

barberi) corn root worm, as well as against the southern (*D. undecimpunctata howardi*) and Mexican (*D. virgifera zeae*) corn root worm. Therefore, a taxonomic differentiation of any given species was included in this text only where relevant.

A total of 112 trials conducted from 1997 through 2001 at different locations across the corn belt had IRDR's of greater than 3.0 in the untreated plots. In these 112 trials, the clothianidin seed treatment averaged a root rating of 2.84 (Iowa scale 1-6) and reduced the root damage significantly compared to the untreated control (IRDR of 4.35). Therefore, clothianidin demonstrated an efficacy equal to the commercial standards chlorpyriphos (IRDR=2.78), and fipronil (IRDR=2.86) applied at full labelled rates. In addition, clothianidin performed at an acceptable level when compared to the highest performing standards tebupirimfos (2.55), and tefluthrin (2.60). All treatments maintained the average IRDR below the threshold IRDR of 3.0 to 3.5 (see Figure 1).

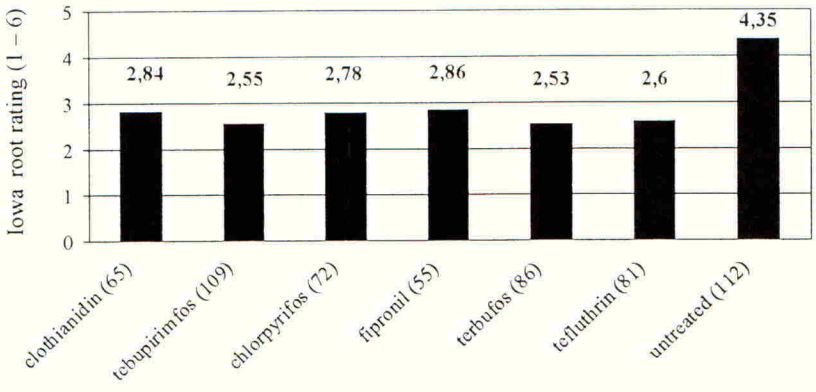

Figure 1. Efficacy of clothianidin seed treatment and commercial soil insecticides on corn rootworms demonstrated by the reduction of root damages (number of trials in brackets)

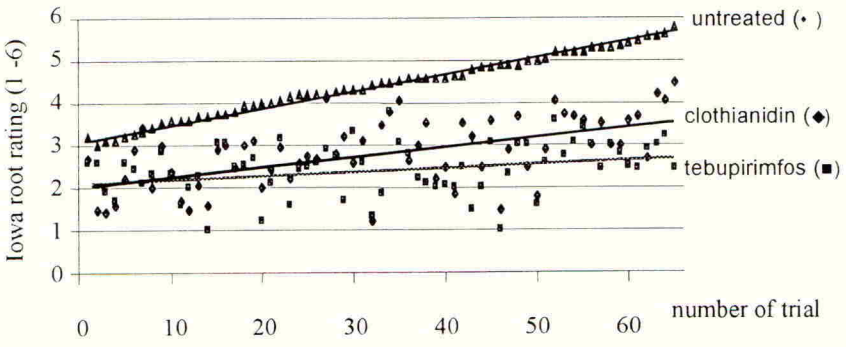

Figure 2. Demonstration of consistency of clothianidin seed treatment and tebupirimfos soil treatment against corn rootworms over all trials

The consistency of the clothiandin performance against corn rootworms becomes obvious from the trial by trial analysis of the results. Only in 26 percent of the roots clothianidin seed treatment resulted in a root rating of IRDR > 3.5: equivalent to organophosphate (18 percent) or fipronil-based soil insecticides (18 percent). The trend from all trials indicates that clothianidin consistently reduces root damage to approximately the factor 1.5 even under extremely severe infestation pressures causing root damages up to IRDR of > 5.0 (see Fig. 2).

Efficacy of clothianidin seed treatment against secondary pests

Clothianidin has also demonstrated a high potential for control of secondary pests of corn. Its spectrum of activity includes: Coleopteran, Lepidopteran, Homopteran, Hymenopteran, as well as Dipteran pests (see Table 1). Due to its systemic properties, clothianidin also affects leaf feeding pests such as chinch bug, corn leaf aphid, and stink bug. Since most of these pests occur early in the season, a high level of efficacy could already be established at very low rates (0.125 – 0.5 mg clothianidin per kernel).

Table 1. Spectrum of activity of clothianidin seed treatment for corn rootworms and for secondary corn pests

Coleoptera:	corn root worm	*Diabrotica spp.*
	wireworm	*Melanotus spp.*
	flea beetle	*Chaetocnema pulicania*
	grape colaspis	*Colaspis brunnen*
	white grub	*Lachnosterna implicata*
Lepidoptera:	black cutworm	*Agrotis ypsilon*
Diptera:	seed corn maggot	*Hylemyia platura*
Homoptera:	corn leaf aphid	*Rhopalosiphum maidis*
Hemiptera	chinch bug	*Blissus leucopterus*
	stink bug	*Nezara viridula*
Hymenoptera	imported fire ant	*Solenopsis spp.*

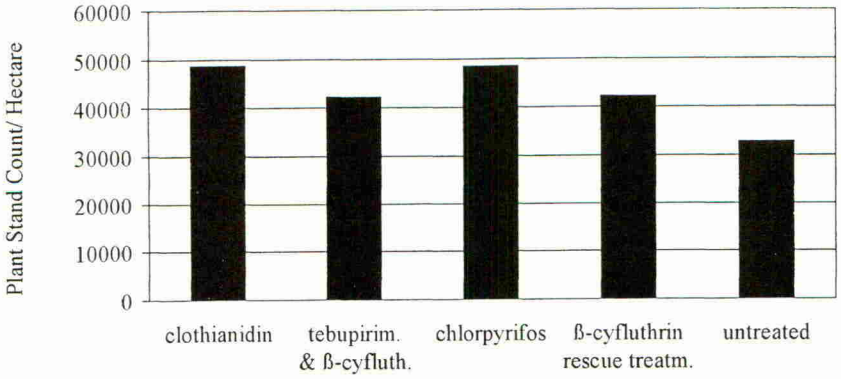

Figure 3. Efficacy of clothianidin seed treatment against black cutworms (*Agrotis ypsilon*) demonstrated by the plant stand count/ hectare

Clothianidin looks also very favourable in its performance against black cutworms (*Agrotis ypsilon*) even at very low rates. The trial results in Fig. 3 show that seed treatment with clothianidin at 0.25 mg a.i./ kernel reduced black cutworm damage and thus maintained plant stand at a level equal to the commercial standard chlorpyriphos applied as an in-furrow granule or ß-cyfluthrin applied as a soil surface rescue spray.

Effect of clothianidin seed treatment on corn yield

Seed treatment with clothianidin always resulted in an excellent plant stand after emergence. The protection against corn rootworm as well as against a wide spectrum of secondary pests consequently led to an increased yield which was demonstrated by four trials under moderate to high infestation levels (see Figure 4). On average, clothianidin at a rate of 1.25 mg a.i./ kernel increased the yield by about 17.6 percent in comparison to the untreated control, and was, therefore, in a similar range as the tebupirimfos granule applications.

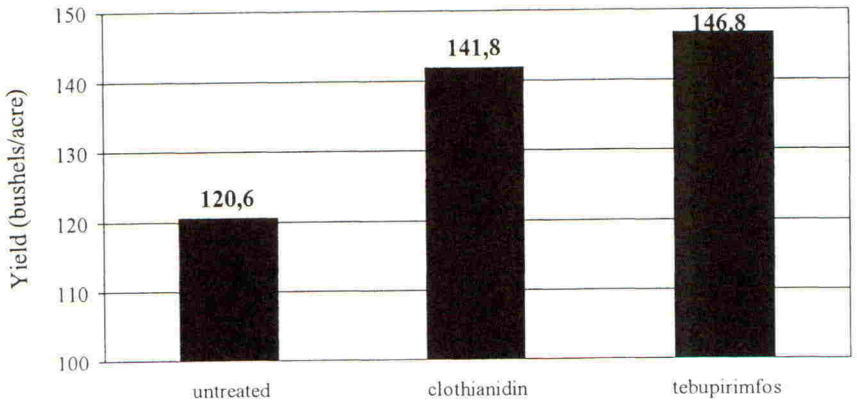

Figure 4. Effect of clothianidin seed treatment on corn yield under moderate to heavy corn rootworm pressure (average from 4 trials)

DISCUSSION

Clothianidin seed treatment successfully controls corn rootworms . At the higher rate of 1.25 mg a.i. per kernel the root damage was reduced on average (IRDR 2.82) to a level significantly below the threshold of IRDR 3.0 to 3.5 and is on an equal level to commercial granular and liquid soil insecticides. Acting primarily after oral ingestion, a feeding damage has to be tolerated so that the larvae become intoxicated with clothianidin and cease feeding immediately. The resulting high level of performance is also confirmed by the consistency rate. Only 26 percent of clothianidin-treated plants had roots with an IRDR > 3.0 to 3.5, which is comparable to competitive organophosphorous (18%) and to fipronil soil insecticides (18%).

Clothianidin is also highly active against most of the secondary pests damaging the seeds or young plants during the early phase after sowing which might be also controlled by soil incorporated insecticides. Other pests, like black cutworm, can be targeted only through soil

surface applications of preventative broadcast treatments or, more commonly, as broadcast rescue treatments. Clothiandin is, therefore, the only substance that can combine control of all early pests at the reduced rate of 0.125 to 0.5 mg a.i./ kernel or secondary pests and corn rootworm at the higher rate (1.25 mg a.i./ kernel) in only one application.

As a consequence of the protection of the seed by clothianidin against early secondary pests from sowing until establishment of the plant stand corn receives an optimal starting base for achieving maximum yield. Protection of the plants against excessive root damage through corn rootworms then ensures the high level of yield, by promoting continuous uptake of water and nutrients (especially under unfavourable growth conditions) as well as by prevention of lodging. This was demonstrated by four trials, which ended up with an improved yield of 17.6 percent in comparison to the untreated control.

Clothianidin permits very flexible pest management in corn production. Historically, corn rootworm has been controlled by crop rotation and use of soil insecticides. Transgenic corn containing *Bt* toxins, etc. are offering an additional tool for combating certain corn pests. Corn varieties resistant to Lepidopteran pests like European corn borer (*Ostrinia nubialis*) are still important in the pest management of corn. Future transgenic corn varieties will also carry Bt-genes for resistance against corn rootworm stacked together with those for control of lepidopterean pests and herbicide resistance. Clothianidin will fit optimally in this integrated pest management program through its potential to control secondary pests from various taxonomic groups. Additionally, clothianidin seed treatment can be used for protection of non-transgenic plants within refuge areas. These areas are being mandated by the Environmental Protection Agency (EPA) to prevent or slow the establishment of pest populations resistant to these Bt-toxins.

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Spirodiclofen: a broad-spectrum acaricide with insecticidal properties: efficacy on *Psylla pyri* and scales *Lepidosaphes ulmi* and *Quadraspidiotus perniciosus*

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ABSTRACT

Spirodiclofen (Envidor) is an IPM-compliant high performance acaricide, which belongs to a new chemical class, the tetronic acids. Its acaricidal mode of action is unique as it interferes with lipid biosynthesis, which is unrelated to other currently registered acaricides. Spirodiclofen shows an excellent and long lasting activity against the main harmful mite species. (Wachendorf *et al.*, 2000).

Besides this acaricidal action, spirodiclofen shows interesting insecticidal activity against pear suckers (*Psylla pyri*) and scale insects (e.g. *Lepidosaphes ulmi, Quadraspidiotus perniciosus)*. A well-timed spirodiclofen treatment, applied at the first main hatching of young orange pear sucker nymphs, provides good control of L1-L3- larvae and inhibits or disrupts the further rapid development to older, dark L4-L5 larvae.

The efficacy of spirodiclofen is less temperature dependent than the current standard amitraz and its long-lasting action is able to protect flowers, fruitlets or shoots for nearly a whole pear-sucker generation. A spirodiclofen-application at the beginning of scale crawler migration also provides excellent control of *Lepidosaphes ulmi* and *Quadraspidiotus perniciosus*. Spirodiclofen shows no adverse effects on natural predators of pear Psylla: e.g. Anthocoridae.

These insecticidal properties enable spirodiclofen to play a key-role in a complementary strategy for the control of pear suckers, the main pest in Western European pear growing. The compound also controls scale insects such as *Lepidosaphes ulmi* (a former secondary pest with increasing significance in pome fruit IPM systems) and *Quadraspidiotus perniciosus*.

INTRODUCTION

Pear sucker is the most important pest of pears and the predominating species in continental Europe is *Psylla pyri*. At least 4 generations occur per year, the first of which has a long and unsynchronized egg-hatching period. This generation is relatively difficult to control due to its long development period, the low presence or absence of predatory bugs (Anthocoridae) at that time and the relatively low temperature in April which compromises the efficacy of amitraz (the current standard). After blossom, several summer generations continue to emerge and can overlap. Their settlement moves from the flower clusters towards the growing shoottips and the underside of apical leaves. Natural occurrence of Anthocoridae contributes significantly to the control of pear suckers from end of May to the beginning of June onwards. In the current control strategy growers rely on the application of amitraz and partially on the ovicidal side effects from fungicides such as maneb and tolylfluanid. Recently, the additional releases of Anthocoridae from reared cultures early in June have been promoted, but results are still too inconsistent to be relied upon.

The mussel scale, *Lepidosaphes ulmi*, is another pest that expands heavily in IPM programmes. Treatment is triggered when the first instar crawlers migrate to the new shoots and fruitlets, usually just after flowering in the second half of May. High populations induce chlorosis and growth inhibition of the tree and fruit damage leads directly to commercial downgrading.

Under Mediterranean conditions, the scale insect *Quadraspidiotus perniciosus* is a polyphagous pest with bad impact on pomefruit and stonefruit. Normally, control of this pest is achieved by applying mineral oil activated with insecticide during winter (till bud burst) followed by the application of a specific insecticide during the migration of the 1st instar of the 1st generation (end of May).

MATERIALS AND METHODS

The insecticidal properties of spirodiclofen were tested in diverse GEP (Good Experimental Practice) field trials, executed according to EPPO guidelines. 12 trials were carried out either on the first generation of pear-sucker, or on the second generation. A randomized block design was used with 4 replicates and 4-6 trees per plot.

All Benelux trials were sprayed with 1000 litres / ha. All compounds were applied 1.5-fold concentrated (with the exception of the Netherlands where a normal concentration was used) when the referring water volume is 1500 litres per 15000 m² leafwall area. In Italy trials were sprayed with 1500 litres / ha. Treatments were carried out with a motorised knapsack sprayer (SOLO Port Type 423). Assessments were done every week along the generation cyclus, respectively on L1-L3 larvae and on L4-L5 larvae on flower clusters and labelled shoot-tips.

Efficacy is calculated according to Abbott's formula. Application rates of spirodiclofen ranged from 0.0096% to 0.0144% a.i. In all trials spirodiclofen, was applied when 30-50 % eggs were close to hatching, characterised by a typical colour change from white to yellow (red eyes of larvae visible) and first L1-larvae presence. Amitraz application was generally delayed for 1 week until there were a higher number of L1- L3 larvae. In order to investigate the stage specificity, two field trials were carried out with application- timings of respectively: newly laid young white eggs, mature yellow eggs and initial hatching, high numbers (>50%) of L1-L3 larvae and at far progressed hatching when large numbers of L4-L5 larvae were present.

GEP - trials according to EPPO were carried out with application on migration of crawlers of *Lepidosaphes ulmi*. *Quadraspidiotus perniciosus* trials on peach were carried out in Italy with assessments on fruits and branches (3*3*3 squares of 4cm² on randomised branches). All data were processed by analysis of variance without transformation. The differences were calculated with LSD based on Student's t-test at a probability value of 0.05 (5%) and by using the Duncan's Multiple range test.

RESULTS

Efficacy on L1-L3 larvae and on L4-L5 larvae of *Psylla pyri*

Spirodiclofen provides a very good control of young L1-L3 larvae of *Psylla pyri* both within the flower clusters and on shoot tips and it performs at least at the same level as the standard amitraz 0.04 % a.i. Young L1 larvae die shortly after hatching and the development towards older larval stages L4-L5 is inhibited or partially disrupted (Tables 1-2).

At 2 weeks after application, a significant reduction of L1-L3 larvae and a significant decrease of the population development towards dark L4-L5 larvae was noticed. The retardation effect of spirodiclofen + surfactant on L4-L5 larvae is in general more obvious than the effect induced by the standard amitraz 0.04%. At 4 weeks after application the overall population reduction obtained by spirodiclofen tends to outperform the standard compound. Tables 1-2 show that the addition of surfactant (polyphenolglycol-ether) enhanced the knockdown activity and also the retardation effect against older L4-L5 larvae.

Table 1. Trial BNL-00-03-210: Number of L1-L3 and L4-L5 stages of *Psylla pyri* (first generation hatching before flowering) and mortality (Abbott) on 10 flowerclusters at 17 and 25 days after application - Application date: 03-Apr-00 (BBCH 56)

Treatment	Date: 20-Apr-00 L1-L3 larvae		Date: 20-Apr-00 L4-L5 larvae		Date: 28-Apr-00 L4-L5 larvae	
	17DAA	Abbott	17DAA	Abbott	25DAA	Abbott
Untreated	58.00 a	-	152.75 a	-	156.75 a	-
amitraz 0.04% a.i.	37.25 ab	35.8	47.00 b	69.2	25.00 c	84.1
thiacloprid 0.0120 % a.i.	46.00 a	20.7	42.25 b	72.3	63.75 b	59.3
spirodiclofen 0.0096 % a.i.	17.00 bc	70.7	24.25 b	84.1	13.50 c	91.4
spirodiclofen 0.0096 % a.i.+ surfactant 0.1%	13.00 c	77.6	14.25 b	90.7	8.75 c	94.4

DAA : Days after application

Table 2.a Trial BNL-00-03-212: Number of L1-L3 stages of *Psylla pyri* (summer generation) and mortality (Abbott) on 25 labelled shoots at 6,15, 22 and 29 days after application - Application date: 31-May-00 (BBCH 73)

Treatment	Date: 06-Jun-00		Date: 15-Jun-00		Date: 22-Jun-00		Date: 29-Jun-00	
	6DAA	Abbott	15DAA	Abbott	22DAA	Abbott	29DAA	Abbott
Untreated	370.00 a	-	163.25 a	-	164.00 a	-	90.25 a	-
amitraz 0.04% a.i.	214.25 bc	42.1	110.50 b	32.3	130.00 ab	20.7	34.75 b	61.5
spirodiclofen 0.0096 % a.i.	264.00 b	28.6	76.75 bc	53.0	44.75 c	72.7	38.75 b	57.1
spirodiclofen 0.0096 % a.i. + surfactant 0.1%	169.25 c	54.3	48.75 c	70.1	29.50 c	82.0	22.75 b	74.8

Table 2.b Trial BNL-00-03-212: Number of L4-L5 stages of *Psylla pyri* (summer generation)and mortality (Abbott) on 25 labelled shoots at 6,15, 22 and 29 days after application - Application date: 31-May-00 (BBCH 73)

Treatment	Date: 06-Jun-00		Date: 15-Jun-00		Date: 22-Jun-00		Date: 29-Jun-00	
	6DAA	Abbott	15DAA	Abbott	22DAA	Abbott	29DAA	Abbott
untreated	62.50 a	-	250.00 a	-	52.50 bc	-	163.75 a	-
amitraz 0.04% a.i.	53.25 ab	14.8	143.75 b	42.5	71.25 ab	-	111.00 b	32.2
spirodiclofen 0.0096 % a.i.	39.75 abc	36.4	110.25 b	55.9	25.50 c	51.4	23.75 c	85.5
spirodiclofen 0.0096 % a.i.+ surfactant 0.1%	27.25 c	56.4	64.50 c	74.2	19.00 c	63.8	21.75 c	86.7

In Figures 1 and 2, the consistency of the good results are shown for spirodiclofen applied at early hatching. It appears that spirodiclofen is more consistent than the reference compound amitraz both regarding its direct action on young larval stages and its retardation effect on dark L4-L5 larvae. The reasons for this are residual effects and the higher temperature independence of spirodiclofen in contrast with amitraz.

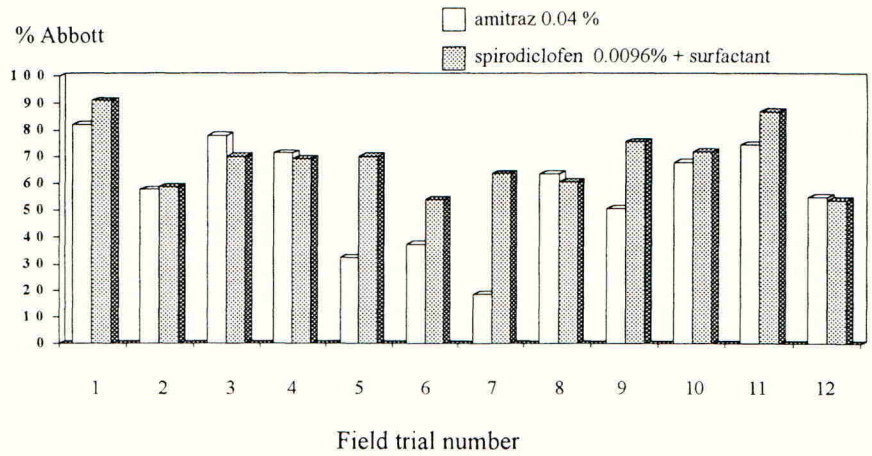
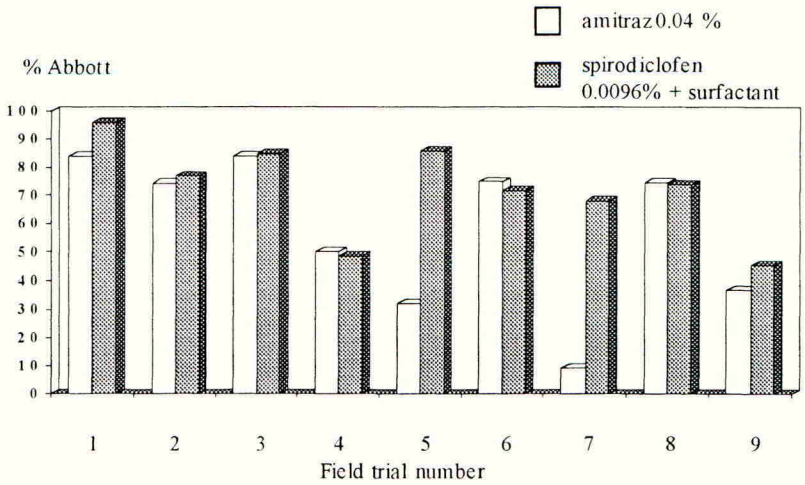


Figure 1. Efficacy of spirodiclofen on young larvae (L1-L3) of *Psylla pyri*:



assessment at 2 weeks after application in 12 field trials in Benelux and Italy

Figure 2. Efficacy of spirodiclofen on old larvae (L4-L5) of *Psylla pyri:* assessment at 3-4 weeks after appl. in 9 field trials in Benelux and Italy

Stage specificity of spirodiclofen on *Psylla pyri*

Figure 3 shows that the optimal application timing for spirodiclofen starts from mature eggs close to hatching and ends when numerous L1 larvae are present. The effect of spirodiclofen on older yellow eggs ready to hatch (orange coloured and red eyes of larvae visible) gave superior results compared to the application on newly laid white eggs of only a few days old. Applied later than 70 % hatching the efficacy of spirodiclofen declines (Figure 3, application at presence of L4-L5). The delay effect on appearance of older L4- L5 is also more pronounced at the early positionings (Figure 4).

Selectivity for Anthocoridae

In pears, spirodiclofen should be selective for Anthocoridae, the main predator for *Psylla pyri*. Both the post-blossom and the crucial midsummer applications were confirmed to be IPM compatible in pears. (De Maeyer et al., 2002)

Efficacy on scales

Spirodiclofen 0.0096% applied at the start of migration of the crawlers (first larval stage) provided excellent control of the scale *Lepidosaphes ulmi*, comparable to the standard phosalone 0.05% (Figure 5). With severe infestations of *Quadraspidiotus perniciosus,* application of mineral oils activated with insecticides before leaf development, is not sufficient to protect the fruits due to the high reproduction potential of this pest; in this case, a specific insecticide application is needed during the migration of the first mobile instars of *Quadraspidiotus perniciosus* at the end of May – beginning of June. The results illustrated in Figure 6 show that spirodiclofen is appropriate for this specific application.

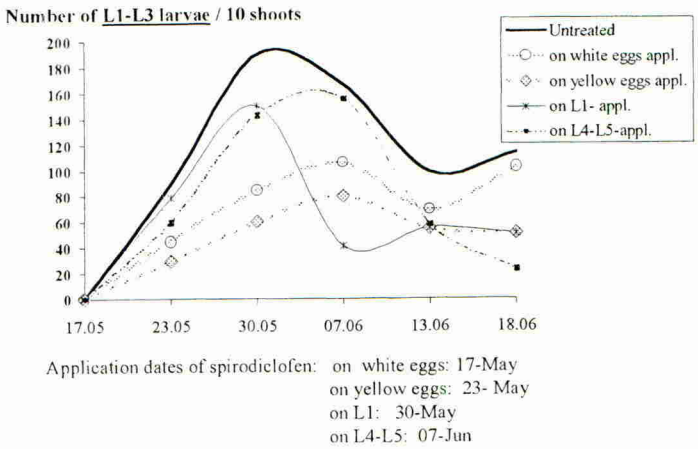


Figure 3. Stage specificity of spirodiclofen on the population dynamics of L1-L3-larvae of *Psylla pyri*

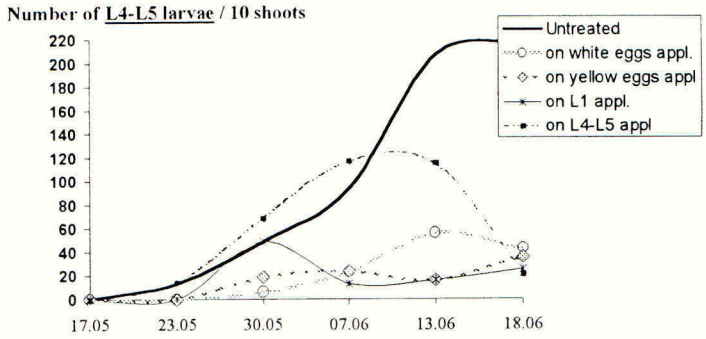


Figure 4. Stage specificity of spirodiclofen on the population dynamics of L4-L5-larvae of *Psylla Pyri*

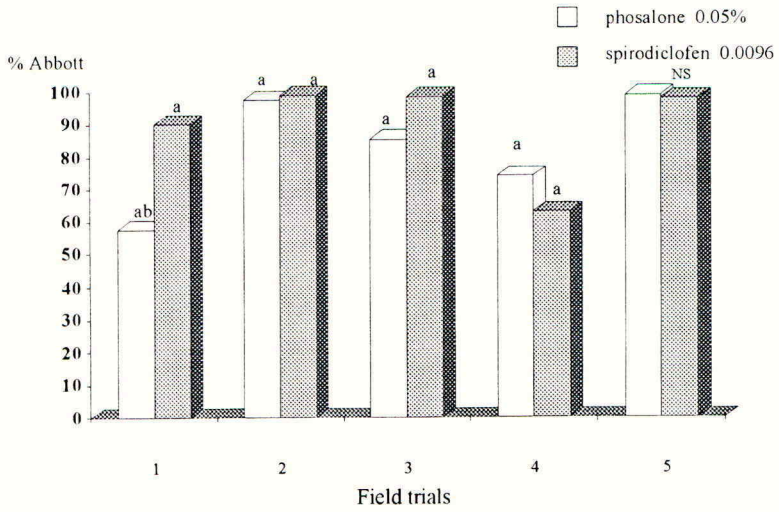


Figure 5 Mortality (Abbott) of *Lepidosaphes ulmi scales* on 50 shoots on 5 trials in Benelux

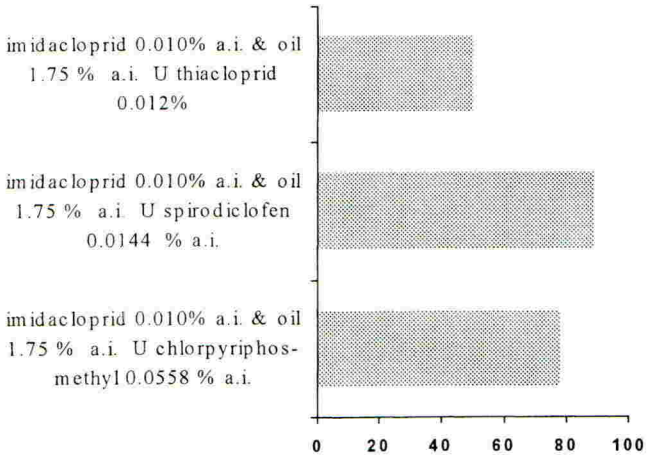


Figure 6. Peach (cv. Baby gold): efficacy against *Quadraspidiotus perniciosus* - Untreated: 44.4 % damaged fruits; application dates: 18.03.01 & 05.06.01.

CONCLUSIONS

Spirodiclofen is highly effective against *Psylla pyri*. Due to its efficacy mainly on young larvae, the application timing is very important in order to control the population waves of young larval stages L1-L3 and additionally disrupt or retard the evolution towards L4-L5 larvae. Spirodiclofen does not depend on high temperature and is therefore more consistent. The optimal application window of spirodiclofen is rather narrow and linked to mature eggs hatching and L1 appearance. A complementary strategy should be implemented in order to combine the specific efficacy of each a.i. on different development stages of this pest: spirodiclofen applied on eggs followed by amitraz 10-15 days later on massive presence of L1-L3 is considered as an option for future pest control. Addition of a surfactant (polyphenolglycol-ether) to spirodiclofen increases generally the efficacy on pear sucker. As polyphenolglycol-ethers will be banned, alternative wetting agents will be required in the future.

Spirodiclofen at 0.0096% is selective on Anthocoridae and fits well in IPM schedules in pears. Applications of spirodiclofen focusing on Psylla or on mites may coincide with migration of either *Lepidosaphis ulmi* or *Quadraspidiotus perniciosus* and are able to reduce infestations of this severe pest. When migration of scales occurs later in time a specific spirodiclofen application on this target is needed.

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