

**The effects of insecticide seed treatments on beneficial invertebrates in sugar beet**

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**ABSTRACT**

A preliminary study investigated the effects of current and novel insecticide seed treatments on the biodiversity of non-target organisms in the sugar beet crop. This trial, conducted at Broom's Barn, Suffolk, during the summer of 2001, compared the novel neonicotinoids, clothianidin and thiamethoxam with two commercial seed treatments, imidacloprid and tefluthrin, and an untreated control. There were no significant effects of insecticide treatments on the number of earthworms, acari or collembola in soil cores, or carabids, staphylinids, spiders and collembola in pitfall traps. Insecticide-treated plots tended to have fewer organisms than untreated plots (though not by a statistically significant margin), especially of predatory arthropods, but this might be an indirect effect of removing herbivorous insects feeding on beet treated with systemic neonicotinoid insecticides. This suggests that long term use of these treatments in a rotation could result in reduction in biodiversity, and indicates that further studies are necessary on a rotational basis.

**INTRODUCTION**

Pest control in sugar beet is currently dominated by the use of imidacloprid seed treatment (Gaucho, Bayer) which was used in 72% of sugar beet crops in 2002. It gives good control of sugar beet soil pests such as springtails, symphylids and pygmy beetles, and foliar pests such as aphids and leaf miners (Schmeer *et al.*, 1990; Dewar *et al.*, 1992; Wauters & Dewar, 1996). Imidacloprid is now also registered as a seed treatment in cereals (Secur) and oilseed rape (Chinook). The potential for continuous use of this product is now therefore high but few published studies have been done to assess its environmental impact in arable crops in field conditions, especially when used frequently in a rotation. This paper describes preliminary results from a study on the effect of imidacloprid, and two new nicotinoid insecticides that are currently under development, on non-target organisms within a sugar beet crop, including earthworms (Lumbricidae), ground beetles (Carabidae), rove beetles (Staphylinidae), spiders (Araneae), springtails (Collembola) and mites (Acari).

**MATERIALS AND METHODS****Treatments**

A field trial was carried out during the summer of 2001 at Broom's Barn, Suffolk. Insecticide treatments were applied as a film coat to pelleted seed at the following rates (given as g a.i. per unit of 100,000 seeds): an untreated control, imidacloprid at 60 or 90 g a.i./unit, tefluthrin at 4 g, thiamethoxam at 60 g, clothianidin at 60 g, a combination of imidacloprid at 60 g and

tefluthrin at 4 g, a combination of thiamethoxam at 60 g and tefluthrin at 4 g, a combination of clothianidin at 60 g and tefluthrin at 4 g, and a 50:50 mix of untreated and imidacloprid coated seed at the commercial rate. The seed was drilled 18.2 cm apart in rows 50 cm apart. Plots were 18 rows by 12 m and the ten treatments were replicated four times in randomised blocks.

## **Observations**

Invertebrates were collected over various sampling periods throughout the trial, using a variety of standard ecological methods. For the purpose of this experiment the samples taken almost immediately post drilling were considered as the pre-treatment sample. At this stage it is very unlikely that any pesticide would have been released from the pelleted seed. Earthworms and carabid beetles were identified to species whereas other groups were identified to family. The numbers of individuals within these species or families was recorded.

### *Earthworms*

Samples were taken from two 0.5 m<sup>2</sup> quadrats per plot on two occasions, the first on 8 May, just after the crop was drilled but before the seed treatments had taken effect, and the second on 4 October at the end of the season. Dilute (0.45%) formalin solution was applied to the soil surface within the quadrat in 10 litres of water. Earthworms that came to the surface during the following 20 minutes were collected and stored in beakers in 10% formalin for identification. The worms were weighed, sorted by length (small, medium and large) and identified to species.

### *Soil organisms*

Three soil cores 15 cm deep by 5 cm in diameter were taken from the centre of each plot. On the first occasion the cores were taken from the holes made for the pitfall traps just after sowing, and on two further occasions by coring close to the pitfall traps. Cores were placed on a modified Tullgren soil extraction machine, and soil organisms collected in 70% alcohol solution. Soil cores were taken on 9 May, 25 July and 3 October.

### *Pitfall trapping*

Three pitfall traps, consisting of a 400 ml plastic beaker, 80 mm in diameter containing 100 ml of 50% ethylene glycol solution as a preservative, were sunk into the ground in plastic sleeves with the top level with the soil surface. Pitfall traps were positioned between rows 10 and 11, three metres from each end and in the centre of each plot. Elevated wooden covers were placed over the pitfalls to prevent rainwater flooding the trap. Traps were set on three occasions of two weeks duration in June, July and August. Invertebrates caught in the traps were sorted and identified as carabids, staphylinids, araneae, collembola and others. Carabids were identified to species.

## RESULTS

### Earthworms

In the pre-treatment sample the most common worm species was *Allolobophora caliginosa* which comprised 89.3% of the total, followed by *A. longa* (5.5%) and *Lumbricus terrestris* (5.2%). At the end of the experiment the total number of earthworms collected had increased over seven-fold, of which *A. caliginosa* was still the most abundant (83.7%), followed by *Lumbricus terrestris* (13%), while *A. longa* was not found. Two new species were identified in the post-treatment sample: *A. chlorotica* (3.3%) and *L. rubellus* (<1%). No significant differences in any species or in the total number of earthworms were found between treatments either at the beginning of the season or at the end (Table 1). Although fewest worms were found in October in plots treated with imidacloprid at 90 g and tefluthrin at 4 g a.i./unit, those plots also had the fewest in May. The proportional change in each treatment was quite variable (range 467-1079%) but not significant.

Table 1. Effect of insecticide seed treatments on the total number of worms/m<sup>2</sup>

Treatment	Rate g a.i./unit	Average total no. worms/m <sup>2</sup>		
		Pre-treatment 8 May	Post-treatment 4 October	Percent change
Untreated		12.3	87.0	707
Imidacloprid	60	11.0	79.8	725
Imidacloprid	90	8.5	59.3	698
Tefluthrin	4	7.5	55.8	744
Imid + Tef	60 + 4	13.8	64.3	466
Thiamethoxam	60	14.3	66.8	467
Thiam + Tef	60 + 4	9.0	86.8	964
Clothianidin	60	9.5	88.3	929
Cloth + Tef	60 + 4	10.0	72.3	723
50:50 unt/imid		9.5	102.5	1079
SED (27df)		3.35	21.81	
LSD (5%)		6.87	44.74	

### Soil organisms

There were no significant effects of any treatment on the number of acari or collembola extracted from the soil cores, either pre- or post treatment, although there were always fewer in the treated plots compared to the untreated plots (Table 2). The very high number of acari recorded in untreated plots on 9 May were mostly found on one plot, indicating the patchy distribution of this group of invertebrates. The reduction in numbers of these organisms in treated plots is not unexpected as the treatments are targeted at soil pests, some of which are collembola.

Table 2. Effect of insecticide seed treatments on soil invertebrates

Treatment	Rate g a.i./unit	Average no. soil pests per treatment					
		Pre-treatment 9 May		Mid season 25 July		Late season 3 October	
		Acari	Collem.	Acari	Collem.	Acari	Collem.
Untreated		321.5	0.5	7.5	7.8	4.0	13.0
Imidacloprid	60	33.3	1.0	4.5	1.8	15.5	5.8
Imidacloprid	90	20.3	0.3	5.3	2.3	7.8	2.5
Tefluthrin	4	52.5	0.0	3.5	0.5	7.8	17.0
Imid + Tef	60 + 4	42.8	6.8	2.8	7.8	5.3	11.8
Thiamethoxam	60	25.8	2.5	3.3	3.3	11.5	5.3
Thiam + Tef	60 + 4	61.3	2.8	4.5	2.3	15.3	6.0
Clothianidin	60	45.0	2.3	2.8	3.3	5.5	8.3
Cloth + Tef	60 + 4	38.5	3.5	5.8	0.0	4.3	7.5
50:50 unt/imid		43.0	0.0	0.3	3.5	8.3	8.8
SED (27df)		121.87	2.86	1.81	3.52	4.37	5.86
LSD (5%)		250.05	5.88	3.71	7.22	8.96	12.03

### Pitfall traps

Over 800 arthropods per plot were caught throughout the sampling period, of which carabid beetles comprised the majority. Staphylinid beetles, spiders and collembola were also fairly numerous.

Twenty species of carabid were found across the trial during June, July and August. The most abundant species was *Pterostichus melanarius*, which comprised 90% of the total, followed by *Trechus quadristriatus* (4.7%), *Harpalus rufipes* (1.5%), *P. madidus* (1.1%) and *Calathus melanocephalus* (1%) (Table 3). No significant differences were found in the cumulative number of carabids caught on the three trapping occasions between treated and untreated plots, although inspection of the data reveals that there were always fewer present in all treated plots than in the untreated plots (Table 4). At the species level, significantly fewer *Bembidion quadrimaculatum* were found in all treated plots than in the untreated plots. However this species comprised less than 1% of the total.

As with carabids, no significant differences were found between the cumulative numbers of staphylinid beetles, spiders and collembola present in the treated plots compared to the untreated plots. However, again there were almost always fewer individuals of each group trapped in the treated plots (Table 4).

### DISCUSSION

This preliminary, one year study has revealed that the insecticides applied as seed treatments to sugar beet, both current and potential future products, have little or no effect on the non-target invertebrates within the soil environment. There were no significant effects on earthworms, acari or collembola in soil cores, carabids, staphylinids, spiders or collembola in pitfall traps. Similar results were found with tefluthrin in an earlier study (Dewar *et al.*, 1990).

Table 3. Proportions of each species of carabid found in pitfall traps.

Species	Cumulative total per plot	Percentage of total	Rank
<i>Agonum dorsale</i>	0.2	<0.1	
<i>Amara apricaria</i>	<0.1	<0.1	
<i>A. eurynota</i>	0.9	0.2	
<i>Bembidion lampros</i>	4.6	0.9	
<i>B. obtusum</i>	0.6	0.1	
<i>B. quadrimaculatum</i>	2.5	0.5	
<i>B. tetracolum</i>	<0.1	<0.1	
<i>Calathus fuscipes</i>	1.2	0.2	
<i>C. cinctus</i>	0.2	<0.1	
<i>C. melanocephalus</i>	5.0	1.0	5
<i>Harpalus affinis</i>	1.5	0.3	
<i>H. rufipes</i>	7.4	1.5	3
<i>Loricera pillicornis</i>	0.1	<0.1	
<i>Nebria salina</i>	0.2	<0.1	
<i>Notiophilus biguttatus</i>	2.0	0.4	
<i>Pterostichus cupreus</i>	0.3	0.1	
<i>P. madidus</i>	5.6	1.1	4
<i>P. melanarius</i>	445.0	88.8	1
<i>Synuchus nivalis</i>	0.2	<0.1	
<i>Trechus quadristriatus</i>	23.4	4.7	2
Total	501.0		

Table 4. Effect of insecticide seed treatments on cumulative number of invertebrates in pitfall traps

Treatment	Rate g a.i./unit	Average no. of invertebrates trapped per treatment			
		Carabids	Staphs	Spiders	Collembola
Untreated		597.8	40.5	127.0	305.8
Imidacloprid	60	492.8	39.0	100.8	224.3
Imidacloprid	90	381.0	30.8	94.5	265.8
Tefluthrin	4	474.5	35.0	104.0	221.0
Imid + Tef	60 + 4	483.5	37.0	98.3	179.0
Thiamethoxam	60	575.3	40.5	97.5	123.0
Thiam + Tef	60 + 4	549.8	38.8	86.3	186.8
Clothianidin	60	482.5	33.5	85.3	160.5
Cloth + Tef	60 + 4	517.5	34.8	98.5	107.5
50:50 unt/imid		450.8	37.8	100.8	256.5
SED (27df)		75.49	10.28	14.74	99.04
LSD (5%)		154.88	21.09	30.25	203.20

There were however consistently fewer of many of these groups in treated plots which, taken over one season may not be important, but could be in the long term, with continuous use of any of the treatments, as might happen with imidacloprid. The slight reductions in the number of the predatory groups such as carabids, staphylinids and spiders, may be an indirect response to the lack of prey on the foliage as a result of the systemic activity of the nicotinoid insecticides, including imidacloprid, thiamethoxam and clothianidin. Further work is necessary to establish whether these slight changes may be exacerbated if sugar beet crops are followed by cereals or oilseed rape treated with imidacloprid in the rotation.

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### Spatial pattern in the distribution of pests and yield in an oilseed rape crop: implications for ICM

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#### ABSTRACT

The distributions of insect pests, plant growth and yield in winter oilseed rape crop were studied in relation to the potential value of spatial information in integrated crop management (ICM). *Ceutorhynchus pallidactylus*, the cabbage stem weevil, *C. assimilis*, the cabbage seed weevil, *Meligethes aeneus*, the pollen beetle and *Dasineura brassicae*, the brassica pod midge, were sampled on a grid and their spatial distributions mapped. Distributions and interrelationships were analysed using Spatial Analysis by Distance Indices (SADIE) and regression. Patterns of *C. pallidactylus*, *C. assimilis* and *M. aeneus* were more complex than hitherto reported, showing differing irregular patches of aggregation, whereas *D. brassicae* was edge-distributed. Stem boring larvae of *Psylliodes chrysocephala* and *C. pallidactylus* caused significant loss of yield and influenced its irregular distribution. The patchiness of *Meligethes aeneus* infestation probably caused spatial variation in the rate of plant maturation, some plants shedding seed before pods on others were ripe. Spatial variability of insect numbers in the crop was partly explained by variation in growth stage. More sophisticated patterns of pest sampling are needed to model crop loss. There is potential for spatially-targeted applications of insecticide and for manipulation of the spatial population dynamics of insects to minimise damage and to optimise the influence of biocontrol agents.

#### INTRODUCTION

The spatial relationships between insects and crop plants remains an aspect of pest / host-plant ecology which has received insufficient attention and the potentially complex spatial interactions of the insect community in oilseed rape with plant growth are poorly understood. The characteristics of spatial heterogeneity of pest populations at a crop scale have implications for sampling and decision-making in integrated pest management. Spatial information is also needed for studies of insect movements into and within crops to underpin the development of targeted pest control strategies (Murchie *et al.*, 1999; Winder *et al.*, 1999;

Ferguson *et al.*, 2000). Few studies of the spatial distributions of pest insects within oilseed rape are based on data from two-dimensional arrays of spatially referenced sampling points.

A succession of pests migrate from their sites of diapause to colonise winter oilseed rape in the UK. Larvae of both *Psylliodes chrysocephala*, the cabbage stem flea beetle, and *Ceutorhynchus pallidactylus* (formerly *C. quadridens*), the cabbage stem weevil, tunnel in the stems. The adults and larvae of *Meligethes aeneus*, the pollen beetle, feed on pollen; if the adults infest plants before flowering, they cause bud abscission when they bite into flower buds to feed on the developing anthers. *Ceutorhynchus assimilis*, the cabbage seed weevil, lays eggs into young pods and its larvae eat the developing seeds. *Dasineura brassicae*, the brassica pod midge, also lays eggs into pods, their larvae consume the tissues of the pod walls and cause them to split prematurely, shedding seed. Each of these pests is univoltine except for *D. brassicae* which has two generations a year on winter rape.

Here we describe the spatial distributions of these pests in a crop of winter oilseed rape and seek evidence for their interaction with the spatial distribution of plant growth and yield.

## MATERIALS AND METHODS

All samples were taken within a 6.6 ha commercially-grown crop of winter oilseed rape (cv. Apex) in Bedfordshire, UK, in 1995. Thirty-six sample locations were defined in an approximately rectangular array with standard minimum distances of 43.5 m between rows and columns and 8 m from sample locations to the crop boundary. No insecticide was applied.

Adult *Ceutorhynchus* spp. were sampled at each location from 20 April (10% flowers open, G.S. 4.1; Sylvester-Bradley, 1985) to 11 July (most seeds brown, G.S. 6.5 - 6.7) using yellow flight traps at crop canopy height (Murchie *et al.*, 1997). Traps were baited with two host-plant volatiles, 2-propenyl isothiocyanate and 2-phenylethyl isothiocyanate.

Counts of insect larvae infesting plants, assessments of insect injury and plant growth, and measurements of seed and oil production were made from plant samples taken at a radius of 5 m from the flight trap at a sub-set of 19 of the 36 sample locations. The measurements made are summarised in Table 1. Numbers of *C. assimilis* larvae and numbers of split pods were assessed in a sample of 20 mature plants taken on 3 July (G.S. 6.3 - 6.5) to the north of each sample location. The sampling dates for *M. aeneus* larvae (18 May) and first generation *D. brassicae* larvae (7 June) were chosen to coincide with the times that they were expected to reach their peak numbers in the crop. Measurements of plant density and yield were made on 11 July in 1 m<sup>2</sup> quadrats. The stems of a sub-sample of 20 plants from each quadrat were dissected and a 'stem injury coefficient' (the ratio of the length of stem with tunnels to the overall plant height above the ground) was calculated as a measure of the severity of tunnelling injury caused by larvae of *P. chrysocephala* and *C. pallidactylus*. At mid-flowering on 9 May, growth stage was recorded at all 36 sample locations.

The spatial distributions of sampled variables were represented as contour maps. Spatial distributions of insect counts were analysed and compared using Spatial Analyses by Distance IndicEs (SADIE; Perry, 1998). The influence of growth stage and plant density on pest numbers and insect injury was tested by multiple regression analyses using forward stepwise selection. Similar regression analyses were used to test the influence of pests on yield.



## RESULTS

The larvae of *D. brassicae* were strongly edge-distributed (Fig. 1a) but each of the other pest species showed a more complex and irregular pattern of clustering (Figs. 1b-e). Analysis by SADIE of adult *C. assimilis* distribution indicated strong aggregation, evidence for more than one cluster and the presence of pattern at a small-scale. The distributions of *C. assimilis* adults and larvae were strongly spatially associated (Figs. 1b & e). No spatial pattern was detected by SADIE for adult *C. pallidactylus* (Fig. 1c), possibly because few insects were caught. Numbers caught of adult *D. brassicae* and *M. aeneus* were too low for SADIE analysis.

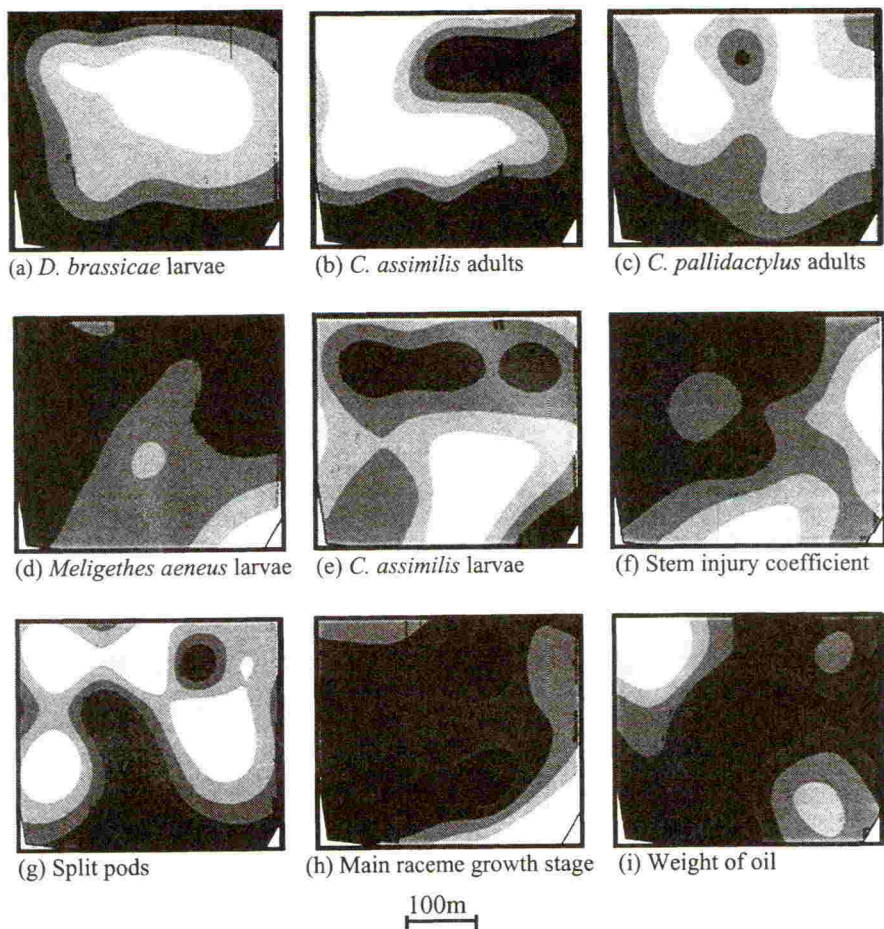


Figure 1. Distribution maps of insects, insect injury, plant growth stage and yield interpolated from samples taken at 19 locations. Each map has seven value classes with contours equally spaced on a logarithmic scale ( $\log_{10}$ ) except for 'stem injury coefficient' and 'main raceme growth stage' which are on a natural scale. Classes with larger values are represented by darker shades. For units of measurement and mean values see Table 1.

Table 1. Mean (SEM) of measurements taken from a subset of 19 of the 36 sample locations.

Total no. of <i>C. assimilis</i> adults per trap	18.4	(4.29)
Total no. of <i>C. pallidactylus</i> adults per trap	3.2	(0.59)
No. of <i>M. aeneus</i> larvae per m <sup>2</sup>	221	(17.4)
No. of <i>D. brassicae</i> larvae per m <sup>2</sup>	650	(139.8)
No. of <i>C. assimilis</i> larvae per 400 pods	35.7	(2.86)
Stem injury coefficient (see text)	0.32	(0.011)
Growth stage (% pods >20 mm on main raceme)	55.3	(3.62)
Plant density (no. of plants per m <sup>2</sup> )	70.1	(5.08)
No. of split pods per 40 racemes	237	(8.1)
Weight of seeds (g / m <sup>2</sup> )	397	(16.3)
Weight of oil (g / m <sup>2</sup> )	184	(8.1)

Table 2. Regression analyses: the influence of plant growth on insect infestation and the influence of insect infestation on yield.

Response variable	Explanatory variables	Estimated regression coefficient (SE)		P	% variability accounted for
No. <i>C. assimilis</i> adults per trap	Intercept	52	(14.2)	0.002	21
	Slope for main raceme growth stage	-0.60	(0.248)	0.026	
No. <i>C. pallidactylus</i> adults per trap	Intercept	10.4	(2.32)	< 0.001	31
	Slope for main raceme growth stage	-0.069	(0.032)	0.048	
	Slope for plant density	-0.048	(0.023)	0.054	
No. <i>D. brassicae</i> larvae per m <sup>2</sup>	Intercept	1902	(436)	< 0.001	31
	Slope for main raceme growth stage	-23	(7.6)	0.008	
No. <i>M. aeneus</i> larvae per m <sup>2</sup>	Intercept	81	(56.8)	0.170	23
	Slope for main raceme growth stage	2.5	(0.99)	0.021	
No. split pods per 40 racemes	Intercept	284	(23.5)	< 0.001	16
	Slope for no. of <i>M. aeneus</i> larvae	-0.21	(0.101)	0.052	
Weight of seeds (g / m <sup>2</sup> )	Intercept	657	(94.5)	< 0.001	27
	Slope for stem injury coefficient	-823	(296)	0.013	
Weight of oil (g / m <sup>2</sup> )	Intercept	318	(46.0)	< 0.001	30
	Slope for stem injury coefficient	-425	(144)	0.009	

Regression analyses revealed that numbers of adult *C. assimilis*, adult *C. pallidactylus*, and *D. brassicae* larvae ('response variables') were all inversely related to main raceme growth stage ('explanatory variable'), whereas numbers of *M. aeneus* larvae were positively related to it (Table 2). However, in each case, the proportion of variation explained was small (21 - 31%).

Numbers of *C. pallidactylus* adults were inversely related to the density of plants (Table 2) but the latter did not significantly influence other measures of insect numbers or injury.

The stem injury coefficient was the only measure of pest populations or pest damage which explained a significant amount of the variation in the yield variables, weight of seeds and weight of oil, and it was inversely related to each (Table 2). A comparison of maps of the stem injury coefficient and the weight of oil (Figs. 1f & i) suggests that this inverse relationship led to complimentary patterns of distribution, i.e. oil yield was lower in areas of the crop where stem injury was more severe. The regression model implies that a stem injury coefficient of 0.32, the mean value recorded, was associated with a 42% (SE 8.7%) loss of oil yield relative to the potential in the absence of stem injury.

The number of split pods was inversely related to the number of *M. aeneus* larvae (Table 2) and examination of the maps of these two variables again suggests their spatial distributions were complimentary (Figs. 1d & g). Although the numbers of split pods and of *D. brassicae* larvae were positively correlated ( $r=0.40$ ), the relationship between them was not significant and their spatial distributions did not appear to be closely related (Figs. 1a & g).

## DISCUSSION

The distributions of *C. assimilis*, *C. pallidactylus* and *M. aeneus* revealed by sampling on a regular grid across a crop were more complex than was apparent from earlier studies of these insects using simpler arrays of sampling locations, i.e. there were differing irregular patterns of aggregation rather than simple edge effects. By contrast, *D. brassicae* larvae showed a simple edge distribution, as predicted from samples taken along line transects into crops. Regression analysis suggested that spatial variability in plant growth in the crop accounted for less than a third of the pattern in pest infestation, implying the influence of unmeasured spatially dependent variables (e.g. wind direction or the location of overwintering sites) or of intrinsic spatial dependence (e.g. an adaptive tendency for the insects to aggregate).

Stem injury caused by larvae of *P. chrysocephala* and *C. pallidactylus* was associated with significant losses in seed number, seed mass and oil mass. There was evidence that the spatial distribution of seed loss was related to that of stem injury, suggesting that there may be potential for spatial targeting of insecticide treatments.

High populations of *M. aeneus* larvae were associated with a decrease in the loss of seed through pod splitting, an unexpected relationship probably due to the delayed maturation of plants infested by *M. aeneus*. The positive relationship between numbers of *M. aeneus* larvae and growth stage suggests that adults had arrived early in plant development relative to their preferred growth stage and sought the most mature plants for oviposition. Plants infested early (at green bud stage) lose the most mature flower buds on their main racemes and may compensate for this injury by the later production of more flowers on axillary racemes. As a consequence, plants infested by *M. aeneus* are likely to complete pod maturation later than uninfested plants and to lose a smaller proportion of their seed by pod splitting. Any spatial pattern of infestation by *M. aeneus* is therefore likely to result in spatial pattern in the rate of plant maturation within the crop. This could lead to harvest being delayed (Lerin, 1987) and some plants shedding seed whilst the pods on others are still ripening. Thus yield losses may be influenced not only by the severity of *M. aeneus* injury, but also by its distribution.

Decision support systems for the control of *M. aeneus* risk underestimating the effect of infestations on crop yield unless they employ sampling strategies which incorporate spatial information.

A better understanding of the spatial population dynamics of pests and their interactions with patterns of plant development and productivity would create opportunities for more precise pest control and reduced insecticide use. There is potential for insecticide to be spatially targeted, reducing the amounts used and reducing the impact on beneficial insects (Ferguson *et al.*, 2000; Warner *et al.*, 2000). Understanding the interplay between the environmental and behavioural factors which determine the spatio-temporal distributions of pests and beneficial insects could lay the foundations for 'push-pull' strategies (Miller & Cowles, 1990) which incorporate not only spatially-targeted insecticides but also spatially-targeted semiochemicals.

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**The use of baited and unbaited traps to monitor the orange wheat blossom midge, *Sitodoplosis mosellana* and its parasitoid, *Macroglanes penetrans***

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### ABSTRACT

Sticky traps have been used to estimate the risk from the orange wheat blossom midge, *Sitodoplosis mosellana* in fields of wheat. Yellow sticky traps were more attractive than blue ones, providing a more accurate assessment of oviposition in the field. A tentative action threshold of 10 adult midges per yellow sticky trap is proposed. Water and sticky traps baited with volatile chemicals given off by wheat plants have been tested for their attractiveness to wheat blossom midge. None of the chemicals tested individually was attractive to the midge, but phenylacetadlehyde attracted increased numbers of the parasitoid *Macroglanes penetrans* to the traps.

### INTRODUCTION

A serious outbreak of damage by larvae of the orange wheat blossom midge (*Sitodoplosis mosellana*) affected UK wheat crops in 1993 (Oakley, 1995). Consequently a considerable area of the UK crop was sprayed with organophosphate or pyrethroid insecticides to control the pest in 1994 (Garthwaite *et al.*, 1995). Incidence declined for a period under this heavy insecticide pressure but has subsequently increased to again cause widespread concern in 2001. Farmers and agronomists have had considerable difficulty in applying the recommended action thresholds based on direct observation of midges laying eggs in crops in the evening (Oakley, 1995). The short time-scale available for observation makes it impossible to cover all the fields that may be at risk and many infestations have been overlooked. Due to the difficulties of assessment and missed infestations, many farmers have considered it necessary to adopt a prophylactic approach to spray application, although the efficacy of such sprays, if not timed to take account of the biology of the pest in the field, may be generally poor (Oakley, 2000). An improved assessment method is required by the industry to minimise any environmental impact and improve the efficacy of the treatments applied.

Oakley *et al.*, (1998) used water traps to measure adult midge activity as part of a prototype forecasting system. Water traps are difficult to use for non-entomologists, as the orange colour of the adult midges is soon lost and considerable taxonomic expertise is required to sort the catch of various flies and other insects. Unbaited sticky traps are easier to use and preserve and present the insects in a more suitable condition for non-specialists to recognise, but still catch many other insects. A trapping system that is more selective in attracting wheat blossom midge could be easily assessed and offer a more accurate assessment of numbers in the field.

Experiments have been conducted to establish the reliability of unbaited sticky trap catches in predicting the level of *S. mosellana* oviposition in wheat. Preliminary experiments have been conducted to establish the attractiveness of some wheat volatiles to *S. mosellana* and its parasitoids.

## MATERIALS AND METHODS

### Unbaited sticky traps

Unbaited sticky traps were tested in ten fields in the summer of 2001. Five of the fields were located at ADAS Bridgets in Hampshire and five at ADAS Boxworth in Cambridgeshire. Traps were 260 mm by 100 mm in standard bright yellow and blue. A total of five pairs of traps were located at 10 m intervals along a tramline within each field. Each pair consisted of a yellow and blue trap, positioned 1 m into the field and 1 m apart. The traps were suspended vertically from canes and positioned just above crop height at GS 53 and removed one week later. When the traps were collected sub-samples of five ears of wheat were taken from between the traps at each point. The bulk sample of 25 ears was dissected under a binocular microscope and the number of *S. mosellana* eggs found on each ear was recorded. The numbers of wheat blossom midge caught on both sides of the trap were recorded and the numbers of other insects caught were estimated by counting those within a 50 mm by 50 mm area in the centre on both sides of the trap. Regression analyses between the trap catches and egg numbers were carried out by MINITAB.

### Baited water traps

#### Experimental design

Responses of *S. mosellana* and *M. penetrans* to traps baited with individual wheat volatiles were compared to the response to unbaited control traps in two field trials in winter wheat in 2000 and one trial in each of winter and spring wheat in 2001 at Rothamsted Farm. In each experiment, a single row of yellow water traps, representing one replicate of a Latin square design (3x3 in 2000 and 4x4 in 2001; Smart *et al.*, 1997), were placed 10 m apart in the crop just prior to the sensitive growth stage GS 55 (Tottman and Broad, 1987) and just below canopy height. The traps were re-randomised within the same row to the next replicate of the Latin square design every 5-7 days and captured insects were removed and identified and counted in the laboratory. Total trap catch data were transformed by  $\log_{10}(x + 1)$  and ANOVA performed. Where appropriate transformed means were compared by least significant difference (LSD) test. Means were then back transformed and are given in the results.

#### Traps

The traps were polypropylene bowls, (111 mm diameter x 49 mm high), painted yellow (ICI autocolor BS381, BS0409) and three-quarters filled with 0.1% aqueous detergent solution. Each trap was mounted in a carrier consisting of a ring of plastic pipe of similar dimensions, also painted yellow, to one side of which was attached a smaller pipe (20 mm diameter x 120 mm long) that fitted over a sectional metal pole. The chemical lure was attached to a plastic plant label that was glued to the side of the carrier, and hung over the bowl.

## Lures

Compounds (Table 1) were obtained from commercial sources and were released individually by diffusion from polyethylene dispensers. Undiluted liquids were applied to pieces of cellulose sponge that were heat-sealed into polyethylene tubing. Nominal release rates were measured in the laboratory at 20°C and 0.5m/s airflow.

Table 1. Compounds tested in field trapping trials

	Release rate (approximate mg/day)
2000	
Benzaldehyde	2.0
Phenylacetaldehyde	10.0
2001	
Phenylacetaldehyde	1.7
$\beta$ -caryophyllene	2.2
Octanal	2.0

## RESULTS

### Unbaited sticky traps

The yellow sticky traps caught 2.4 times as many wheat blossom midges as the blue traps (Figure 1). The yellow trap catches gave a significant regression ( $P = 0.019$ ,  $R^2 = 51.7\%$ ) in predicting egg numbers of:

$$\text{egg numbers} = 3.01 (\pm 2.46) + 0.229 (\pm 0.078) \text{ mean 5 yellow trap catch.}$$

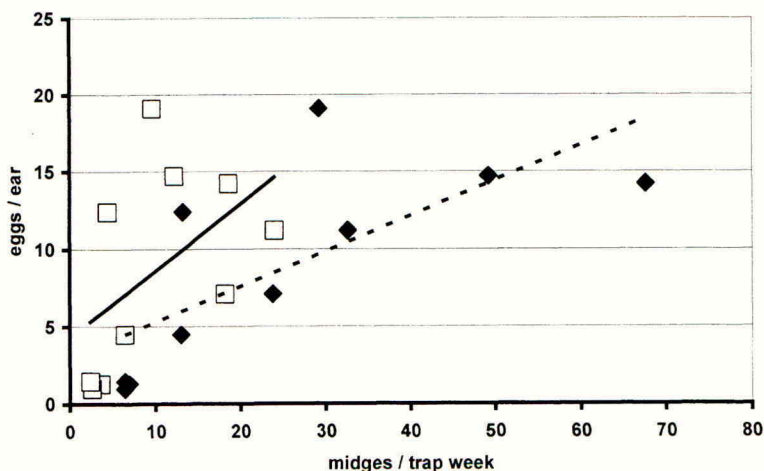


Figure 1. Catches of midges on yellow (◆—) versus blue (□—) traps compared to numbers of eggs laid on ears.

Catches from a random selection of two yellow trap catches per field were analysed to test whether five traps gave an improved fit over two. The two traps gave a similar regression ( $P = 0.008$ ,  $R^2 = 60.1\%$ ):

$$\text{egg numbers} = 0.95 (\pm 2.62) + 0.320 (\pm 0.092) \text{ mean 2 yellow trap catch.}$$

The catches from the blue traps gave a poorer regression with the egg numbers ( $P = 0.130$ ,  $R^2 = 26.2\%$ ):

$$\text{egg numbers} = 4.30 (\pm 3.21) + 0.431 (\pm 0.255) \text{ mean 5 blue trap catch.}$$

The yellow traps caught more insects than blue traps, with *S. mosellana* representing 0.19% of the total catch, which averaged 2600 insects per trap. On blue traps there were on average 1500 insects per trap, of which 0.14% were *S. mosellana*.

### Baited water traps

In 2000, the majority of midges and parasitoids were caught between 7-28 June, and as there was no significant difference between the two trials the results of a combined ANOVA are presented in the Tables. A total of 234 *S. mosellana*, mostly females, were caught in the trials, with approximately even numbers between the treatments and the control traps and no significant difference between treatments (Table 2). A total of 1889 parasitoids were captured over this period, 98% in the phenylacetaldehyde baited trap  $P < 0.001$  (Table 2).

Table 2. Back transformed mean numbers of *S. mosellana* and *M. penetrans* caught in baited yellow water traps 7-28/6/00.

	<i>S. mosellana</i>	<i>M. penetrans</i>
Blank	12.3	4.1
Benzaldehyde	10.5	1.8
Phenylacetaldehyde	10.9	166.3
<i>P</i>	NSD	< 0.001

In 2001, the period of highest trap catches was 1-19 June on the winter wheat site. A total of 88 midges, were caught but there were no significant difference between treatments. Over the same period 5493 parasitoids were caught, approximately 99% in the phenylacetaldehyde baited trap,  $P < 0.001$  (Table 3). Most midges and parasitoids were caught between 14-28 June on the spring wheat site. A total of 84 midges were trapped, but there were no significant difference between treatments. A total of 15,778 parasitoids were caught, 97% in the phenylacetaldehyde treatment  $P < 0.001$  (Table 4).

Table 3. Back-transformed mean numbers of *S. mosellana* and *M. penetrans* caught in baited yellow water traps 1-19/6/01 in winter wheat.

	<i>S. mosellana</i>	<i>M. penetrans</i>
Blank	3.8	4.4
Phenylacetaldehyde	2.7	880.3
$\beta$ -caryophyllene	4.8	3.5
Octanal	5.2	6.0
<i>P</i>	NSD	< 0.001



Table 4. Back-transformed mean numbers of *S. mosellana* and *M. penetrans* caught in baited yellow water traps 14-28/6/01 in spring wheat.

	<i>S. mosellana</i>	<i>M. penetrans</i>
Blank	1.9	28.7
Phenylacetaldehyde	5.2	1827.8
$\beta$ -caryophyllene	5.5	18.1
Octanal	4.7	21.3
<i>P</i>	NSD	< 0.001

## DISCUSSION

The study with unbaited sticky traps has demonstrated the potential for trapping systems to aid in decision making on the need to treat fields against *S. mosellana*. On the basis of this study it has been provisionally proposed that two sticky traps should be deployed per field with an action threshold of 10 *S. mosellana* per trap. The large number of other insects caught by these traps could easily confuse inexperienced observers and this method would probably only be suitable for experienced agronomists. ADAS consultants used unbaited sticky traps to aid decisions on wheat blossom midge control in 2002. Comments were generally favourable with the traps identifying that no action was not required in the fields examined. There were problems with identification at sites where no *S. mosellana* were present, when other similar insects were more easily confused. Ear samples were checked from eight fields where counts were below threshold and a maximum of 4.5 eggs per ear was found in a field with a mean trap catch of eight *S. mosellana*. This level of infestation would have been insufficient to cause significant yield loss.

The additional attractiveness of yellow compared to blue traps was not expected and may indicate that the adult midges use colour as part of their orientation towards suitable hosts. A greater accuracy in predicting egg numbers, resulted from this moderate level of attraction. If a suitable chemical attractant can be found to bait a less attractive trapping system, such as a Delta trap, greater precision could be obtained whilst reducing the numbers of other insects caught.

The female sex pheromone, 2,7-nonanediyl dibutyrate, has been identified by Gries *et al.* (2000). This pheromone could be used to attract male midges to traps. Such a system could be used to provide an early warning of oviposition, as the males fly to mate with the female midges before seeking suitable host plants on which to lay their eggs (Pivnick & Labbé, 1992 & 1993). As oviposition flights can involve movement between fields to find a crop at a suitable growth stage for oviposition, considerable re-distribution of females may occur. The numbers of males caught by a pheromone trap in a given field may therefore not give an accurate indication of the degree of egg laying in a crop.

None of the individual plant volatiles tested here proved to be attractive to the midges and work continues to identify active compounds. Phenylacetaldehyde attracted increased numbers of the parasitoid *Macroglanes penetrans*. This parasitoid is one of the principal natural enemies of *S. mosellana* in the UK as well as in Canada (Doane, *et al.*, 1989). Parasitoid activity may not be proportional to midge oviposition, and is unlikely to be directly useful in a decision support system. However, the attractiveness of phenylacetaldehyde to *M. penetrans*

could be exploited in an integrated control strategy in order to concentrate the parasitoids in non-insecticide treated areas.

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**Observations on integrated population management strategies for wheat bulb fly**

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**ABSTRACT**

This study set out to investigate the feasibility of achieving long-term suppression of wheat bulb fly (*Delia coarctata*) populations on a co-ordinated whole-farm basis. A combination of novel and conventional control methods were employed in the study, including the use of trap fallows as decoy egg-laying sites (using set-aside whenever possible) and the selective use of insecticides targeted against adult flies at their emergence sites in wheat during the summer. The population control measures were applied on the project farm (c. 1000 ha) in Cambridgeshire and compared with a nearby reference farm (c. 500 ha), on which no population management was adopted. The area of trap fallow used each year ranged from 0.8 to 40.9 ha. Water traps were used to monitor adult fly activity during the summer and to guide the application of insecticides targeted against adult flies at high-risk emergence fields in July, before the flies dispersed to egg-laying sites. There was some evidence that the population control strategy adopted at the project farm contributed to the decline in wheat bulb fly egg numbers at this site. In autumn 2000, egg numbers at the project farm decreased compared with the previous year, whereas numbers at the reference farm and in the regional forecast increased. The proportion of the wheat area at risk of attack with egg numbers greater than the 2.5 million eggs/ha threshold also declined from 49% in 1997 to 8% on the project farm in 2000. In 2001, there was an overall decline in egg numbers at all sites and although numbers on the project farm remained lower than the reference farm, a change in crop rotation on the project farm meant that a smaller area of wheat remained at risk of attack as fewer wheats were sown after crops such as potatoes and sugar beet. The trap fallows were not successful as greater numbers of eggs were laid on the soil surface beneath the canopy of sugar beet and potato crops and the possible reasons for this are discussed. The observed effects of the population control strategy may thus have been associated with the impact of the summer sprays targeted against the adult flies at their emergence sites, but further work is required to verify this.

**INTRODUCTION**

Wheat bulb fly (*Delia coarctata*) is a serious pest of wheat and is endemic in the eastern counties of the UK, particularly in East Anglia and in Lincolnshire, Humberside and Yorkshire. The flies emerge in June and lay their eggs on bare soil, in the absence of a host crop, during July and August. Egg laying takes place in fallows, after early harvested crops such as vining peas, or beneath the canopy of crops such as onions, sugar beet and potatoes.

The eggs remain dormant until the following winter, when they hatch and larvae invade wheat plants during January, February and March. The larvae invade just below soil level and feed within the stems causing the appearance of withered and yellow central shoots known as 'deadhearts'. As the larvae feed and grow, they move between shoots or between plants so that crop damage increases. Wheat crops sown after onions, sugar beet, potatoes and vining peas are frequently attacked and late-sown crops are particularly vulnerable owing to their early growth stage at the time of invasion. Once the larvae are fully fed, they leave the plants to pupate in the soil in May, completing one generation each year (Young & Ellis, 1996).

The objective of this study was to investigate the feasibility of a strategy to achieve the long-term suppression of wheat bulb fly populations on a whole-farm basis. If successful, such an approach could lead to savings in the annual chemical costs of controlling the pest as well as reducing the potential environmental risks of insecticide use. Long-term suppression of wheat bulb fly populations can only be brought about by an integrated control strategy to reduce the year-on-year survival of the pest over a wide area. Adult wheat bulb flies can migrate up to 0.8 km to lay their eggs in bare soil during the summer (Bardner *et al.*, 1968), so in order to minimise the inward migration of flies from surrounding farmland a control programme was implemented on a large farm estate of approximately 1000 ha, near Ely, Cambridgeshire.

A combination of novel and conventional control methods were employed in the study. These included trap fallows (using set-aside whenever possible) and the selective use of insecticides targeted against adult flies at their emergence sites in wheat during the summer and against larval infestations in wheat during the winter. The study reported here covers observations made over a five year period from 1997 to 2001.

## **MATERIALS AND METHODS**

A large farm estate (c. 1000 ha) near Ely, Cambridgeshire, was chosen as the project farm. The farm is located on the fen and comprises of a mixture of organic peat and mineral soils and the crop rotation included wheat, sugar beet, potatoes and onions. A reference farm (c. 500 ha), with a similar crop rotation to that of the project farm, was selected in the Littleport area, approximately ten miles away from the project farm. No population control measures were implemented at the reference farm as this site was intended to be compared with the population control measures adopted at the project farm. No restrictions were placed on the normal use of insecticides against wheat bulb fly at either farm, which proceeded each year according to local commercial practice. However, at both farms, the results of the wheat bulb fly egg population assessments done each autumn were made available to the host farmers, which enabled them to target insecticide use to high-risk wheat fields containing egg numbers in excess of the action threshold of 2.5 million eggs/ha.

### **Trap fallows**

Trap fallows were created by cultivation to expose uncropped bare soil before the wheat bulb fly egg laying period in July and August each year, 1997–2001. Freshly cultivated soil with a rough surface is known to be an attractive egg-laying site for the pest (Young & Talbot, 1996). Eggs are laid on the exposed soil of the trap fallows which are subsequently sown with a non-host crop (i.e. non-cereal) in the autumn, so that when the eggs hatch during the following

winter the larvae will die in the absence of a suitable host plant. Therefore, the trap fallows serve as decoy areas for the egg-laying flies (Young & Ellis, 1996). The size and location of the trap fallows varied each year according to the availability of suitable areas on the farm. Wherever possible, whole fields of rotational set-aside were designated as trap fallows. The set-aside regulations permitted cultivation of set-aside after July 1<sup>st</sup> and the trap fallows were created by the host farmer by ploughing, which, in most cases, took place in the first week of July (Table 1).

Table 1. Trap fallow numbers, areas and cultivation dates at the project farm 1997–2001.

Year	Number of trap fallows	Total area of trap fallows (ha)	Cultivation dates
1997	8	0.8	21 May & 5 July
1998	8	2.6	1 June & 1 July
1999	4	34.8	1–7 July
2000	3	40.9	1–7 July
2001	8	7.5	1–7 July

### Monitoring adult wheat bulb fly emergence and flight

The emergence and activity of adult flies was monitored each summer at the project farm with water traps. Each year, water traps were placed in a range of emergence and egg-laying sites at the project farm (Table 2). Each trap comprised of a white plastic tray (33 cm long × 23 cm wide × 5 cm deep) filled with water to a depth of about 3 cm. A small amount of detergent was added to the water to assist in the wetting of trapped insects. The water level was maintained in the traps by the use of reservoir water bottles attached to each trap. The traps were emptied and re-set at weekly intervals during June, July and August. The trap catches were sieved in muslin in the field and were then stored in 70% ethyl alcohol prior to examination and counting under a low-power microscope in the laboratory.

Table 2. Water trapping regimes at the project farm, 1997–2001.

Year	Number of water traps deployed	Number of fields with water traps	Trapping period
1997	6	2	17 June – 26 August
1998	10	10	30 June – 18 August
1999	12	12	16 June – 18 August
2000	12	12	7 June – 23 August
2001	16	10	20 June – 22 August

### Control of adult flies at emergence sites

It may be possible to reduce wheat bulb fly population carry-over by controlling female flies whilst they remain in the wheat crop at their emergence site, before they disperse to lay eggs

in fields destined to grow wheat the following season (Young & Ellis, 1996). Water traps were used to monitor the activity of wheat bulb flies on selected fields where large numbers were expected to emerge. These traps were also used to indicate the optimum time to apply insecticide sprays to kill adult flies whilst they remained at their winter wheat emergence fields. The fields selected for summer sprays were those which had experienced high levels of wheat bulb fly damage during the previous winter and were, therefore, expected to harbour large numbers of adult flies during the summer. A synthetic pyrethroid insecticide (normally lambda-cyhalothrin), approved for use against cereal aphids in wheat, was applied to selected fields each July by the host farmer using commercial application equipment at the rate recommended for use against cereal aphids (Table 3). The sprays were also applied in the evenings, to coincide with the flight activity of the adults (Bardner *et al.*, 1977).

Table 3. Insecticide sprays applied against adult wheat bulb fly in emergence fields at the project site, 1997–2001.

Year	Insecticide and formulation	Rate/ha	Spray date	Nos. of fields sprayed	Area sprayed (ha)
1997	Alpha-cypermethrin, 100 g/l EC	150 ml	9 July	2	21
1998	Lambda-cyhalothrin, 50 g/l EC	100 ml	3 July	5	52
1999	Lambda-cyhalothrin, 50 g/l EC	100 ml	6 July	7	60
2000	Lambda-cyhalothrin, 50 g/l EC	100 ml	7 July	4	40
2001	Lambda-cyhalothrin, 50 g/l EC	100 ml	11 July	7	80

### Assessing and forecasting egg numbers

Egg numbers in the trap fallows and all other fields prone to wheat bulb fly egg-laying were assessed in the autumn of each year at the project farm. A range of representative fields were also sampled at the reference farm each autumn. Each field sample comprised of 32 soil cores each of 7.5 cm diameter. Smaller samples comprising of 16 cores per sample were taken when sampling small areas of trap fallow (< 0.5 ha). The depth of sampling ranged from approximately 10 cm to 25 cm and was adjusted according to the depth of cultivations since the start of egg laying. Wheat bulb fly eggs were extracted using a modified Salt and Hollick (1944) technique involving a standard process of soaking soil samples in water followed by separation in water, wet-sieving, and flotation in magnesium sulphate solution. The number of fields sampled each year ranged from 25 to 41 at the project farm (including trap fallows) and from 13 to 17 at the reference farm (Table 4). Forecasts of wheat bulb fly numbers were also calculated each autumn using the predictive equations developed by Young & Cochrane (1993) to give an overall indication of the expected annual trends in egg numbers.

## RESULTS

The numbers and activity of male wheat bulb flies peaked in mid-July, whilst peak female activity each year was approximately two or three weeks later. An example of this annual pattern is shown in Figure 1. Based on these observations, the peak egg-laying period occurred from mid-July to mid-August. Consequently, the sprays against the adult flies were

applied from the 3<sup>rd</sup> to the 14<sup>th</sup> of July (Table 2), before the main dispersal and egg laying period was judged to occur. Although the water traps were not deployed to assess the impact of the sprays applied against the adult flies, there were no discernible effects of the spray treatments on the numbers of wheat bulb fly caught.

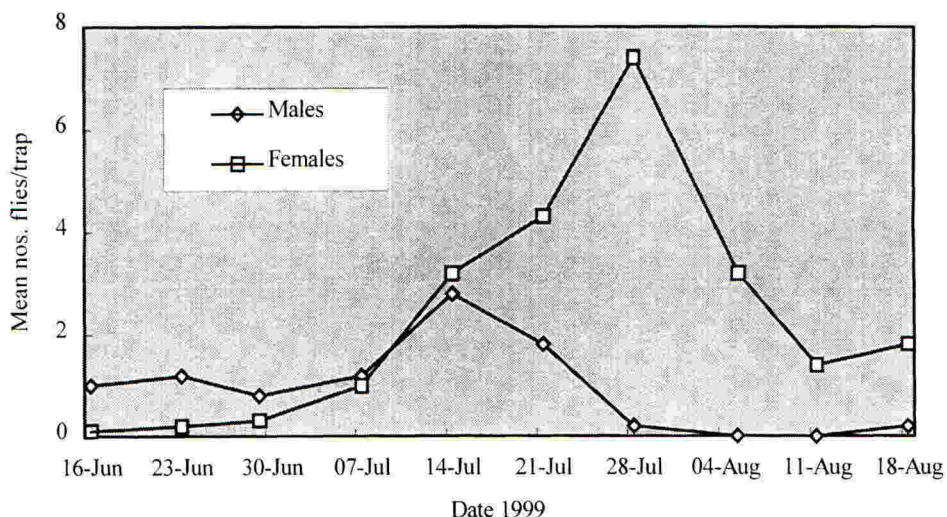


Figure 1. Water trap catches of adult wheat bulb fly on the project farm during 1999.

Egg numbers in the trap fallows on the project farm were disappointingly low in all years and more were laid on the soil surface beneath the canopy of sugar beet and potatoes (Table 4). In autumn 2000, egg populations increased at the reference farm compared to 1999 and this upturn was also reflected by the regional forecast. In comparison, at the project farm, the reverse trend was observed and egg numbers declined in 2000 compared with the previous year (Table 4, Figure 2). In 2001, the forecast predicted a downturn in egg numbers, a trend which was repeated at both farms but egg numbers remained lower at the project farm than at the reference farm.

The total area of wheat grown on the project farm remained relatively stable until 2001 when, owing to changes in crop rotation, the wheat area at risk of wheat bulb fly attack declined substantially (Table 5). The area of wheat grown in fields with wheat bulb fly egg populations in excess of the action threshold of 2.5 million eggs/ha also declined over the study period and was notably lower in autumn 2000, at a time when the regional forecast and the reference farm indicated that egg numbers were higher than in the previous two years (Figure 2, Table 5). At the start of the study in 1997, 49% of the total wheat area at risk of attack on the project farm was grown in fields containing egg numbers in excess of the threshold (Table 5). However, in 2000 this proportion declined to 8%, which was against the general trend of higher egg numbers observed at the reference farm and predicted by the regional forecast. Insecticide costs (Table 5) declined at the end of the study in 2001 but this was linked mainly to changes in crop rotation which reduced the area of wheat grown in fields at risk of attack (e.g. after potatoes and sugar beet).

Table 4. Wheat bulb fly egg numbers at the project and reference farms, 1997–2001. Figures in parentheses indicate the number of fields sampled.

Year and site		Mean egg numbers (millions/ha) and crops sampled					
		Potatoes	Sugar beet	Trap fallows	Others*	Overall mean	Overall SEM
1997	Project farm	3.2 (13)	4.7 (12)	0.8 (8)	4.9 (8)	3.4 (41)	0.40
	Reference farm	3.6 (4)	4.0 (4)	na	2.9 (5)	3.5 (13)	0.70
1998	Project farm	1.9 (11)	1.8 (17)	0.8 (8)	2.3 (4)	1.7 (40)	0.20
	Reference farm	1.5 (5)	0.8 (2)	na	1.9 (7)	1.4 (14)	0.25
1999	Project farm	1.5 (4)	2.5 (15)	0.5 (4)	3.5 (2)	2.0 (25)	0.26
	Reference farm	0.8 (3)	1.9 (3)	na	2.7 (8)	1.8 (14)	0.35
2000	Project farm	1.8 (8)	1.4 (22)	0.7 (3)	2.5 (4)	1.6 (37)	0.17
	Reference farm	2.1 (3)	3.4 (3)	na	3.5 (8)	3.0 (14)	0.75
2001	Project farm	1.0 (14)	1.3 (17)	0.2 (8)	na	1.0 (39)	0.13
	Reference farm	1.0 (6)	1.7 (5)	na	1.5 (6)	1.4 (17)	0.20

\* Includes onions, peas, set-aside and lettuce.

Table 5. The annual areas of wheat at risk of wheat bulb fly attack and associated insecticide use against wheat bulb fly on the project farm, 1997–2001.

Year	Total wheat area (ha)	Wheat area at risk of attack (ha)	Wheat area above threshold <sup>1,2</sup> (ha)	Wheat area treated with insecticide <sup>3</sup> (ha)	Insecticide costs (£)
1997	390	241	119 (49)	249	1810
1998	395	184	47 (26)	101	1407
1999	344	134	54 (40)	99	976
2000	350	206	16 (8)	133	1684
2001	341	99	0 (0)	38	474

<sup>1</sup> 2.5 million eggs/ha.

<sup>2</sup> Data in parentheses shows wheat area above threshold as a percentage of wheat area at risk.

<sup>3</sup> Including multiple applications and excluding sprays against adult flies.

## DISCUSSION

There was some evidence that the population control strategy adopted at the project farm contributed to the decline in wheat bulb fly egg numbers at this site. The strongest effect was observed in autumn 2000, when egg numbers at the project farm decreased compared with the previous year, whereas numbers at the reference farm and those predicted by the regional forecast increased. In 2001, there was an overall decline in egg numbers and egg numbers on the project farm remained lower than the reference farm. Additionally, a change in crop rotation meant that a substantially smaller area of wheat was at risk of attack in 2001 because less wheat was grown after potatoes and sugar beet.



The failure of the trap fallows to attract large numbers of eggs is difficult to explain. Predation of eggs by ground beetles is considered unlikely to have resulted in the consistently low egg numbers found in the trap fallows. It would appear that the more humid and sheltered microclimate beneath the canopy of sugar beet and potato crops presented a more favourable egg-laying site than the exposed fallows at the project farm. Earlier studies have indicated that fallows can be highly attractive egg-laying sites. For example, Young & Talbot (1996) reported egg numbers as high as 10.9 million eggs/ha (1088 eggs/m<sup>2</sup>) in fallowed plots. Therefore, the poor performance of the trap fallows at the project farm may be linked with site-specific conditions and should not be taken as a general dismissal of trap fallowing as a potentially useful cultural control technique.

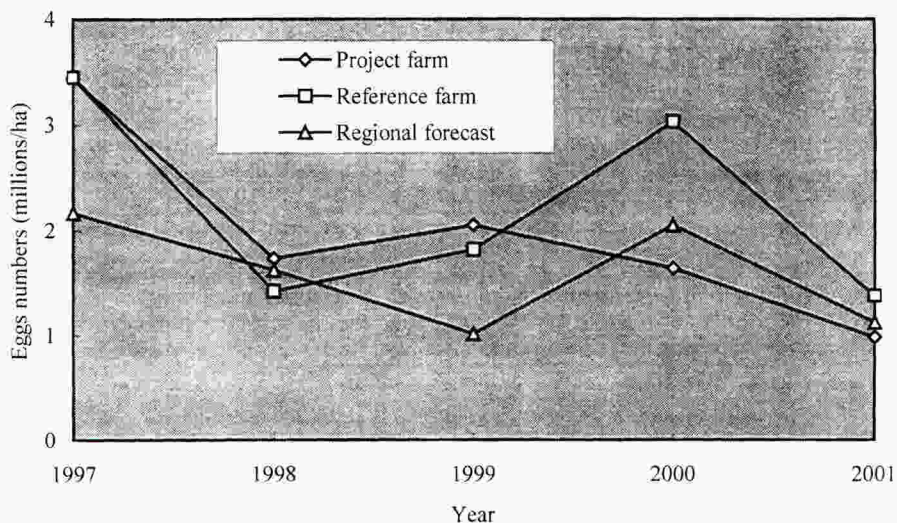


Figure 2. Mean numbers of wheat bulb fly eggs at the project and reference sites compared with the annual regional forecast.

In the absence of a strong effect from the trap fallows, it is possible that the observed impact of the population control strategy was associated with the summer sprays targeted against the adult flies at their emergence sites. In this study, it was not possible to adopt methods to quantify the direct effects of the summer sprays on the numbers of adult flies but the results suggest that further work is required to verify the impact of these treatments. However, the widespread use of insecticides against adult wheat bulb flies would be inappropriate and environmentally undesirable. If insecticides are to be employed against adult flies, their use would need to be closely managed as part of a whole-farm strategy and targeted selectively to emergence sites where field observations show that high infestations exist. Future research could usefully focus on developing environmentally benign methods of controlling adult flies. For example, parasitic fungi (*Entomophthora* spp.) are known to attack adult wheat bulb fly. Wilding & Lauckner (1974) considered that these fungi were important regulators of wheat bulb fly populations, which suggests that fungal pathogens might usefully be exploited in future as biological control agents.

The commercial costs of wheat bulb fly control on the project farm declined during the study period from 1997 to 1999 but they were higher than expected in 2000. This was because one of the wettest autumns on record for the Ely area delayed the sowing of wheat after late-lifted root crops. Therefore, the late-sown wheat in autumn 2000 was judged to be vulnerable to attack and a high proportion of the area at risk received a wheat bulb fly seed treatment. Although the annual spend on wheat bulb fly insecticides on the project farm was substantially lower by the end of the study in 2001, this reduction could not be attributed solely to the impact of the population control measures because the area of wheat at risk of attack, sown primarily after potatoes and sugar beet, declined in 2001 owing to changes in crop rotation.

The integrated, whole-farm, approach to controlling wheat bulb fly populations in this study has shown promise. The long-term control of wheat bulb fly populations in this way is likely to be improved if the immigration of wheat bulb flies from surrounding farmland can be reduced by co-ordinating and extending the population control area to include neighbouring farms.

#### ACKNOWLEDGEMENTS

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**Protecting oilseed rape from slug damage using metaldehyde, methiocarb and imidacloprid seed dressings**

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**ABSTRACT**

Slugs are serious pests of oilseed rape that readily kill very young seedlings and thus could potentially be controlled by seed dressing. The molluscicides metaldehyde and methiocarb and an insecticide containing imidacloprid and beta-cyfluthrin were tested as seed-dressings in the laboratory. Metaldehyde and methiocarb were effective seed-dressings and reduced slug damage whereas the insecticide did not. However, when the two molluscicides were tested in a semi-field experiment, only the methiocarb seed dressing caused a significant reduction in slug damage. In addition, slug damage was recorded in three field experiments in which a range of insecticides was being tested as seed treatments for oilseed rape. In one experiment at a site with few slugs present, slug damage was significantly reduced by imidacloprid + beta-cyfluthrin and methiocarb seed treatments compared with the control plants. No significant effects on slug damage were seen in the remaining two field experiments.

**INTRODUCTION**

Slugs are serious pests of oilseed rape, causing most damage immediately after emergence when seedlings are often fatally damaged thus reducing plant stand (South, 1992; Moens & Glen, 2002). Over recent years slug damage to oilseed rape has increased greatly mainly due to changes in agronomic practices. The increase in slug damage to oilseed rape is mirrored in the increased usage of molluscicides in this crop. In 1994 120,829 hectares of oilseed rape were treated with molluscicidal baits in the UK. By 2000 this figure had risen to 195,059 hectares (M Thomas personal communication).

Slugs are currently controlled by broadcasting slug bait pellets on the soil surface which does not always protect crops adequately, even when multiple applications are made. Since rape is only at risk during germination, emergence and first leaf stages (Moens and Glen, 2002), it may be possible to protect the crop by seed dressing, a strategy that offers both environmental and economic benefits.

Previous work on seed treatments to control slug damage has focussed on protecting cereal seeds as opposed to seedlings, with the most promising chemicals being metaldehyde (Ester *et*

*al.*, 1996; Nijënstein and Ester, 1998) and methiocarb (Scott *et al.*, 1984; Ester and Nijënstein 1995). However, work by Nijënstein and Ester (1998) has shown that coating wheat seeds with molluscicides protects not only the seeds, but also the emerged seedlings.

The objective of this study was to investigate the potential of commercially-available molluscicides to be used as seed dressings for oilseed rape. In addition, we aimed to determine whether a range of developmental or commercially-available compounds that were being considered as possible insecticidal treatments to replace lindane (gamma-HCH) in oilseed rape had any influence on slug damage. One of these insecticides (imidacloprid) has previously been shown to reduce slug damage to wheat seeds (Rose & Oades, 2001).

## MATERIALS AND METHODS

### Laboratory bioassays

#### Comparison of metaldehyde, methiocarb and imidacloprid seed dressings

Oilseed rape seeds (cv. Apex) were coated with either metaldehyde (70 g a.i./kg seed) or methiocarb (23 g a.i./kg seed) mixed with a commercial seed adhesive, 'Sepiret' (Agrichem, Whittlesey, UK), as described in Simms *et al.* (2002). Control seeds were coated with seed adhesive only. Imidacloprid was applied to seeds as 'Chinook' (imidacloprid and beta-cyfluthrin) at 20 ml/kg seed, by Bayer. Laboratory bioassays were set-up as detailed in Simms *et al.* (2002), with sixty treated seeds sown in half seed trays with four field-collected adult *Deroceras reticulatum* (mean biomass = 1.8g/tray SE 0.04g). Seed trays were placed in a cold frame covered by a shading net. All experiments had fully randomised designs, with six replicates. Four weekly assessments of slug damage and mortality were made after planting.

#### Comparison of seed dressings with slug pellets

Oilseed rape seeds (cv. Pronto) were dressed with metaldehyde (58g a.i./kg seed) or methiocarb (18g a.i./kg seed) and tested against metaldehyde slug pellets ('Metarex Green', 6% metaldehyde, recommended application rate 8 kg product/ha, De Sangosse, UK) and methiocarb slug pellets ('Draza', 4% methiocarb, recommended application rate 5.5 kg product/ha, Bayer, UK). Seeds for slug pellet treatments and controls were coated with seed adhesive only. Seed trays were prepared and planted as described above. Slugs were added immediately after sowing. Slug pellets were added at the manufacturer's recommended dose, according to the area of the seed tray, three days after sowing. The experiment had a fully randomised design, with six replicates.

### Semi-field experiment – mini-plots

The outdoor experiment was set up in mini-plots in an experimental area at IACR-Long Ashton. Each mini-plot is a plastic container (80 cm x 60 cm (0.48 m<sup>2</sup>) x 23 cm deep, filled with loam soil to 20 cm depth, with drainage holes in the bottom covered by plastic mesh to prevent slugs entering or leaving. Plots are surmounted by copper-mesh fences 10 cm high to deter slug movement between plots. Seeds (cv. Pronto) were sown in each plot (48 seeds per plot). The design was a randomised block with treatments of either metaldehyde dressed seeds, methiocarb dressed seeds, metaldehyde slug pellets (8 kg product/ha) and control.

seeds, methiocarb dressed seeds, metaldehyde slug pellets (8 kg product/ha) and control. Seeds were treated as described above. Ten adult slugs (*D. reticulatum*) were then introduced to each plot of the four treatments described above. In addition, no slugs were added to one plot in each block, which contained two rows of control seeds and one row of seeds with each molluscicide treatment. This additional plot provided a check on seed germination in the absence of slugs as well as a check on whether slugs were able to move between plots. Plots were irrigated daily. Numbers of plants damaged by slugs were recorded weekly for 5 weeks.

### Field experiments with test insecticides

In harvest year 2001, three plot-scale trials were drilled. In Staffordshire and Hampshire, sites were drilled on 25 and 29 August 2000 respectively. In Warwickshire, the site was drilled deliberately later on 13 September 2000 at a site selected primarily on the basis of slug risk. All trials were drilled at a 4.5 kg seed/ha with the objective of providing 50-55 plants/m<sup>2</sup>.

Commercially-available winter oilseed rape cv. Pronto was pre-treated with the fungicides iprodione + thiram (2.5 + 3.0 g a.i./kg seed) which served as control seeds. These were overtreated with the test insecticidal or molluscicidal seed treatments. Active ingredients included imidacloprid (at 2.0 or 10.0 g a.i./kg seed) + beta-cyfluthrin (2.0 g a.i./kg) and two developmental seed treatments, A and B, at rates selected by the manufacturers. Two additional, commercially-available test compounds were also included in the study. These were thiram and carbosulfan (as TMTD 98% + Combicoat® CBS, SATEC) or thiram and methiocarb (Pomarsol + Mesurol, supplied by CPB Twyford).

A spray treatment of lambda-cyhalothrin (as Hallmark, Syngenta) was applied to additional plots of control seeds and imidacloprid (2.0 g a.i./kg) + beta-cyfluthrin seeds in the late autumn as a test standard for cabbage stem flea beetle (*Psylliodes chrysocephala*). This as an important autumn pest of oilseed rape, formed a further component within the investigation but is not considered further in this paper.

A sample of 50 plants was removed from each plot to assess percentages of plants damaged by slugs at the one-two expanded leaf stage (GS 1.1; 1.2, Sylvester-Bradley, 1985) and to record the incidence of damage to cotyledons and first true leaves. A second sample of 10 plants per plot was taken in December and returned to the laboratory to assess the incidence and type of pest damage.

## RESULTS

### Laboratory bioassays

In laboratory bioassays comparing different molluscicidal and insecticidal seed dressings (Figure 1a), only the molluscicides metaldehyde and methiocarb significantly reduced slug damage to oilseed rape seedlings. Metaldehyde seed-dressing reduced slug damage significantly more than all other treatments, while methiocarb seed-dressing significantly reduced slug damage in comparison with control treatment only (Figure 1a). None of the doses of the insecticidal seed-dressing imidacloprid + beta-cyfluthrin significantly reduced slug damage (Figure 1a). In bioassays comparing seed-dressings with slug bait pellets, all slug control treatments significantly reduced slug damage in comparison with the control

treatments (Figure 1b). In addition, metaldehyde seed-dressing also significantly reduced slug damage in comparison with methiocarb bait pellets (Figure 1b).

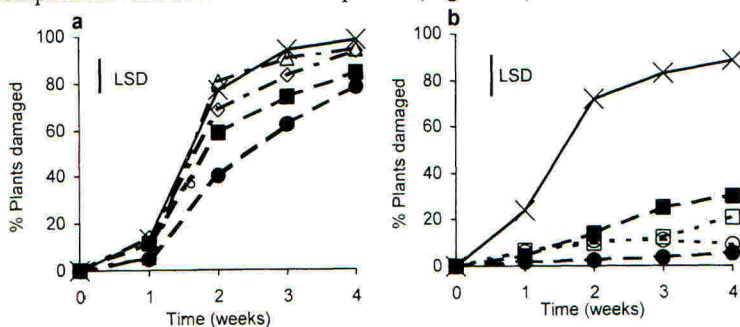


Figure 1. Laboratory studies of efficacy over time of (a) seed dressings: control (X), metaldehyde (●), methiocarb (■), imidacloprid + beta-cyfluthrin (20 ml/kg) (◇) or imidacloprid + beta-cyfluthrin (40 ml/kg) (Δ) and (b) seed dressings vs. bait pellets: control (X), metaldehyde on seed (●), metaldehyde pellets (○), methiocarb on seed (■) and methiocarb pellets (□), (LSD  $P = 0.05$ , (a) 80 df, (b) 100 df).

#### Semi-field experiments – mini-plots

Metaldehyde bait pellets significantly reduced slug damage in comparison with all other treatments, and methiocarb seed dressing significantly reduced slug damage in comparison with metaldehyde seed-dressing and control (Figure 2). All seeds germinated and no slug damage was recorded in plots without added slugs.

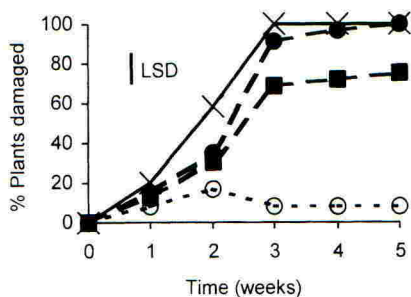


Figure 2. Comparison of efficacy of seed treatments in a mini-plot trial with treatments of either control (X), metaldehyde seed-dressing (●), metaldehyde bait pellets (○), or methiocarb seed-dressing (■) (LSD  $P = 0.05$ , 42 df).

#### Field experiments with test insecticides.

In the Staffordshire trial, significant reductions in mean percentages of plants damaged by slugs were obtained from methiocarb and both rates of imidacloprid + beta-cyfluthrin seed dressings compared with the control mean (30.0% of plants damaged, Table 1), but with no significant differences between these treatments. Significant reductions in percentage area of cotyledons damaged by slugs were also obtained from imidacloprid + beta-cyfluthrin,

carbosulfan and methiocarb compared with the control mean where 1.74% of the cotyledon area was damaged.

In Hampshire, a more severe slug attack was not significantly reduced by any of the test treatments compared with the untreated mean (95.0% damage at GS 1,2). In Warwickshire, where the crop was drilled into a clay soil, a severe slug attack developed which resulted in complete destruction of cotyledons and the first true leaf on many plants. None of the treatments provided significant reductions in slug damage compared with control means (data not shown for the Hampshire and Warwickshire sites).

Table 1. Mean % plants attacked by slugs at GS 1,1-1,2 in Staffordshire

Test Treatment	Mean % plants damaged by slugs	Mean cotyledon % area damaged by slugs
1. Control	30.0	1.74
2. Imidacloprid (2.0 g a.i./kg) + beta-cyfluthrin	14.0 **	0.67 *
3. Imidacloprid (10.0 g a.i./kg) + beta-cyfluthrin	13.5 **	0.29 **
4. Experimental A low rate	20.0	1.37
5. Experimental A high rate	25.0	1.20
6. Carbosulfan	18.5	0.66 *
7. Methiocarb	13.5 **	0.87 *
8. Lambda-cyhalothrin spray	34.0	1.87
9. Imidacloprid (2.0 g a.i./kg) + beta-cyfluthrin + lambda-cyhalothrin spray	13.5 **	0.66 *
10. Experimental B.	19.5	1.05
LSD $P = 0.05$ , 27 df	11.61	0.870
F probability treatment	0.007	0.017

\*, \*\* significantly different from untreated means at  $P = 0.05$  and  $0.01$  respectively.

All seeds were treated with fungicides iprodione and thiram except treatments 6 and 7 that received thiram only.

## DISCUSSION

Both molluscicides, metaldehyde and methiocarb, gave good protection to oilseed rape seedlings under laboratory conditions. This is in general agreement with other workers, who have reported these molluscicides to have great potential as seed-dressings (Scott *et al.*, 1984; Ester *et al.*, 1996; Nijenstein and Ester, 1998; Simms *et al.*, 2002). When compared with bait pellets the molluscicidal seed-dressings protected oilseed rape seedlings as well as pellets. However, in our semi-field conditions the efficacy of metaldehyde and methiocarb seed-dressings was less than in laboratory bioassays and short lived, with only methiocarb seed-dressings showing significant results in mini-plots. This is in contrast to findings of Scott *et al.* (1984) and Ester *et al.* (1996) who found metaldehyde and methiocarb seed-dressings reduced slug damage as well as slug bait pellets under field conditions. The reduction in efficacy of our seed treatments in semi-field trials may be due to the higher slug pressure in this experiment, and/or loss of seed-dressing a.i. due to environmental factors such as rainfall, volatilisation, microbial degradation or reduced uptake at low temperatures.

In this study, imidacloprid was found to reduce slug damage in the field trial conducted in Staffordshire where there were few slugs, but not in laboratory experiments or the other two field experiments. The slug pressure in the laboratory was probably greater than in the Staffordshire site and thus results were more in line with the other two field experiments. It may be that where growers apply imidacloprid and beta-cyfluthrin to oilseed rape, it will confer some slug protection, but this will be insufficient if slug pressure is high.

Both metaldehyde and methiocarb show promise as seed-dressings to control slug damage in oilseed rape. Further investigations are required to identify the constraints to efficacy of molluscicidal seed-dressings in the field. Once these have been detected, new seed dressing formulations could be produced to overcome the constraints thus increasing the efficacy of our seed-dressings. It must also be noted that while seed-dressing can protect seedlings from slug damage, they act as repellents and are not necessarily fatal to slugs in this type of application. It is therefore suggested that molluscicidal seed-dressings may play an important role as part of an integrated pest management system, together with slug bait pellets, reducing the number of bait pellet applications and giving seedlings vital protection at their most vulnerable stages.

## ACKNOWLEDGEMENTS

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### Semiochemicals for the control of cereal pests

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#### ABSTRACT

The potential of three semiochemical treatments, each having a different mode of action, was evaluated in laboratory, semi-field and small plot field trials for use in cereal pest management strategies. The treatments were a) *Tasmannia stipitata*, a plant extract, containing the antifeedant polygodial, b) *cis*-jasmone, a stress related volatile plant activator inducing defence mechanisms within the wheat plant, and c) nepetalactone, an aphid sex pheromone component attractive to aphid parasitoids. Settlement of the grain aphid, *Sitobion avenae*, was significantly reduced on wheat seedlings treated with either *T. stipitata* extract or *cis*-jasmone in laboratory and field simulation experiments. Aphid numbers in field plots were reduced by both *cis*-jasmone and *T. stipitata* treatments, although not significantly. *T. stipitata* was more effective against autumn than spring aphid populations and in addition, reduced numbers of larvae of orange wheat blossom midge, *Sitodiplosis mosellana*, and gout fly, *Chlorops pumilionis*, the latter significantly. Although in semi-field trials, parasitism of *S. avenae* by the parasitoid *Aphidius ervi* was increased significantly on nepetalactone baited plants compared to untreated controls, field populations of parasitoids and predators were too low for statistical analyses. However, numbers of aphids were lower in the nepetalactone plots compared to the controls.

#### INTRODUCTION

Insects use chemical information (semiochemicals) from their environment at all stages of development and particularly to locate acceptable host plants. Many semiochemicals are also involved in multitrophic interactions and influence the behaviour of natural enemies of pests. Thus, semiochemicals provide an opportunity to develop pest management strategies that do not rely on conventional insecticides. In this study the efficacy of semiochemicals with different modes of action was evaluated for cereal aphid control. The treatments comprised: a) an extract of *Tasmannia stipitata*, (Illiciales: Winteraceae), (obtained from B Milgate Australian Native Foods Group) containing the antifeedant polygodial (Basta & Spooner-Hart, 1999; Dawson *et al.*, 1986); b) *cis*-jasmone, a stress related volatile plant activator that influences release of semiochemicals from exposed plants resulting in reduced pest colonisation and increased attraction of beneficial insects, particularly aphid parasitoids (Birkett *et al.*, 2000); and c) nepetalactone, an aphid sex pheromone component derived from the catmint, *Nepeta cataria*, attractive to aphid parasitoids (Glinwood *et al.*, 1999).

## MATERIALS AND METHODS

### Laboratory choice tests (*T. stipitata*)

Individual oat seedlings (cv. Revisor) were sprayed electrostatically at 10.4l/ha with *T. stipitata* extract at 16.4mg/ml (170g/ha) formulated in 10% aqueous ethanol solution. Control seedlings were sprayed with 10% aqueous ethanol. One treated and one control seedling were placed in a ventilated cylindrical glass chamber (17cm high x 11cm diameter) and 10 alate grain aphids, *S. avenae*, were introduced. There were 12 replicate chambers and they were kept at 22±1°C. The number of aphids settled on each seedling was recorded after one, two, five and 24 hours. Settlement on treated seedlings was compared with settlement on controls using a Mann-Whitney 'U' test (Genstat).

### Field simulation assay (*cis*-jasmone)

Fifty wheat seedlings, (cv. Axona, growth stage 11-12, Tottman and Broad, 1987) sown in a 5 x 10 grid of 2 x 2cm square pots, were sprayed hydraulically with *cis*-jasmone, formulated in a 0.1% aqueous solution of a nonionic surfactant Ethylan BV (EBV) (Akcros Chemicals, Manchester, U.K.), at a rate equivalent to 50g /ha in 200l /ha. Control plants were sprayed with 0.1% EBV and were kept separately to avoid contamination. The plants were treated 48 h prior to use in the bioassays to allow sufficient time for the defence metabolism to be altered by the *cis*-jasmone.

Treated and control seedlings were tested under no-choice conditions in a Perspex simulator (90 x 30 x 30 cm, wind speed 0.5 m/s, 22°C, 40% R.H.) on alternate days (n = 8 for each treatment). The wheat seedlings were positioned at the upwind end of the simulator and 250 alate *S. avenae*, which had acclimatised to the conditions of the simulator for 1h prior to the start of the experiment, were released downwind from a Petri dish lined with moistened filter paper. Counts of settled aphids were made two, five and 24 h after release and settlement on treated seedlings was compared with controls using a *t*-test (Genstat).

### Polytunnel experiment (nepetalactone)

Trials were carried out in a simulated wind tunnel (1.5m high x 2m wide x 4m long) within an unheated polytunnel fitted with fans providing a wind speed of 0.5m/s. Five pots of approximately 80 wheat seedlings (GS 11-12) were each infested with 200 apterous *S. avenae* late on the day prior to the test and left overnight in the polytunnel. The following day 40 mated female parasitoids, *Aphidius ervi*, were released downwind of the plants and left for 24h, after which any parasitoids remaining were removed. The plants were put into netting bags and kept in a heated glass house until mummified aphids were discernible (approximately 12 days) when they were counted. Treated and control plants were tested under no-choice conditions on alternate days (n = 8 for each treatment). The nepetalactone lures (a polymer formulation releasing 50µg/day) were attached, one per pot, to sticks at plant height. Control plants were untreated. Transformed data ( $y = \log_{10}(x + 1)$ ) were assessed by *t*-test.

### Field experiment

Plots of winter wheat, cv. Riband (12 x 12m), were sown on 13 September 2000 in a 4 x 4 Latin square design. Treatments were applied on 6 October and 3 November 2000 and 11 May

and 5 June 2001 using a hand-held hydraulic sprayer fitted with Lurmark 015-F110 T-jets delivering 200 litres/ha, at a rate of 50g *cis*-jasmone/ha and 600g *T. stipitata* extract/ha (both formulated in 0.1% aqueous Ethylan BV). The nepetalactone was released from formulated polymer at 200µg/day/plot. Because the EBV formulation had no effect on aphid settling (Birkett, *et al.*, 2000) the control plots were untreated. Cereal aphids, predominantly *S. avenae*, parasitised aphids and any predators present were counted on 100 plants per plot on five occasions between September and November 2000 and on 100 tillers per plot on eight occasions between early May and mid-July 2001. In addition, 64 plants per plot were collected on 15 February to assess for gout fly, *Chlorops pumilionis*, infestation, and 20 tillers per plot were taken on 9 July to assess for wheat blossom midge, *Sitodiplosis mosellana*, larvae. Transformed data [ $y = \log_{10}(x + 1)$ ] were subjected to ANOVA and the means transformed back for the Tables.

## RESULTS

### Laboratory and field simulation studies

In the choice test with the *T. stipitata* extract there were significant differences, for all the observations, between the number of aphids settled on treated and control plants (Table 1).

Table 1. Effect of *T. stipitata* treatment on settlement of *S. avenae* in a choice chamber bioassay

	1 hour count		2 hour count		5 hour count		24 hour count	
	treated	control	treated	control	treated	control	treated	control
Mean	1.0	2.8	1.2	3.4	1.8	4.4	3.0	5.3
S E	0.33	0.61	0.41	0.67	0.46	0.62	0.66	0.62
<i>P</i>	0.029		0.014		0.007		0.025	

After 24 h, wheat seedlings treated with *cis*-jasmone consistently had significantly fewer alate *S. avenae* settled compared to control seedlings ( $P = 0.012$ , Figure 1), representing a 37% reduction in colonisation.

Parasitism of *S. avenae* by *A. ervi* was significantly increased on aphid infested plants baited with nepetalactone compared to untreated controls ( $P < 0.05$ ). The mean number of mummified aphids formed was 185 on treated plants and 92 on untreated plants.

### Field experiment

In field plots, aphid infestation was not significantly reduced on individual sampling dates by any of the treatments, but the overall trend in aphid numbers was lower compared to the control plots. Cumulative aphid numbers in the autumn and the following spring/summer are shown in Table 2. The *T. stipitata* treatment was most effective in the autumn, reducing aphid infestation by 33% compared to control plots. In addition, numbers of larvae of wheat blossom midge and gout fly were reduced, the latter significantly ( $P < 0.01$ , Table 3). Too few

parasitised aphids or predators, predominantly aphidophagous hover fly larvae (Syrphidae), were present for statistical analysis, but of the total numbers seen in the 2001 assessments (Table 4) there was a slight increase for some treatments over controls and no deleterious effects of treatments.

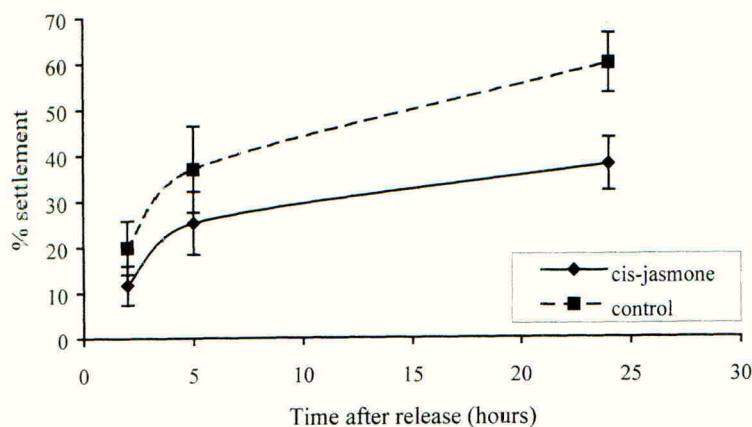


Figure 1. Mean Settlement of alate *S. avenae* on *cis*-jasmone treated and control seedlings in the simulator (error bars indicate SE of mean)

Table 2. Mean cumulative number of cereal aphids on 100 plants/tillers per plot and percentage reduction compared to control

	Control	<i>T. stipitata</i>	<i>Cis</i> -jasmone	Nepetalactone
Autumn 2000	30.5	20.5	21.8	36.0
Mean				
% reduction	-	33	29	+18
Spring/summer	1179	1132	958	908
2001 Mean				
% reduction	-	4	19	23

Table 3. Mean number of gout fly and orange wheat blossom midge larvae in winter wheat, February and July, 2001

	Control	<i>T. stipitata</i>	<i>Cis</i> -jasmone	Nepetalactone
Gout Fly 15/2/01				
Mean no. larvae/tiller*	0.28 a	0.21 b	0.25 a	0.25 a
Midge 9/7/01				
Mean no. larvae/tiller	17.2	13.4	16.5	18.6

\* Values with different letters are significantly different, least significant difference test ( $P < 0.01$ )

Table 4. Total parasitised aphids recorded in 2001 assessments and total syrphid larvae in three assessments between 25 June and 9 July 2001

	Control	<i>T. stipitata</i>	<i>Cis</i> -jasmone	Nepetalactone
Parasitised aphids	155	150	182	170
Syrphid larvae	59	79	74	89

## DISCUSSION

Although all three semiochemical treatments showed highly significant effects in the laboratory and field simulation trials, results were not quite as dramatic under field conditions. This was largely due to patchy aphid distribution in the autumn and high population pressure in spring/summer. The reduction in aphid colonisation observed in the laboratory and the field with the *T. stipitata* extract supports results obtained in earlier experiments (Dawson *et al.*, 1986), in which incidence of Barley Yellow Dwarf Virus was reduced by application of an extract of *Polygonum hydropiper*, a UK plant also containing polygodial. In the latter trials, three electrostatic applications of the *P. hydropiper* extract to winter barley were required to achieve effective aphid control. However, the *T. stipitata* extract contains substantially more polygodial than the *P. hydropiper* extract (37%, compared to approximately 17% in the purified extract of *P. hydropiper*, H B Rassmussen personal communication) allowing the use of a conventional hydraulic sprayer and a reduction in the number of applications. Optimal application rates for the *T. stipitata* extract were determined in the laboratory trials using electrostatic application and then corrected for use with conventional hydraulic sprayer (B J Pye personal communication). Although the data for aphids were not significant in the field, there was a reduction in their number in the autumn when control, particularly for BYDV, is most important (Oakley & Young, 2000). Indeed, the greatest use of insecticides on cereal crops is of autumn applied pyrethroids for BYDV control.

Aphid parasitoids are a key group of aphid natural enemies maintaining pest populations below economic levels. However, a number of studies have highlighted the importance of having high numbers of parasitoids in the crop in the autumn to establish sufficient overwintering populations to provide effective aphid control in the following spring. The lack of parasitoids in autumn 2000, presumably associated with the unusually wet and windy conditions, impacted on the *cis*-jasmone and nepetalactone treatments, designed to manipulate natural populations of these key beneficial insects. Nevertheless, although the field data were not significant, the trend of lower populations in the *cis*-jasmone treated plots agreed with earlier experiments where aphid numbers were lower and significant reductions were observed. In addition, the overall higher numbers of parasitised aphids in these plots confirms experiments showing increased parasitoid activity on *cis*-jasmone treated plants (Birkett *et al.*, 2000; Bruce *et al.*, in preparation). Similarly, the field simulation trials confirmed earlier experiments with natural parasitoid populations showing that parasitism could be significantly increased by nepetalactone. However, the field trials were inconclusive showing only slight increases in total numbers of parasitised aphids and aphidophagous syrphid larvae.

Autumn application of *T. stipitata* had unexpected effects against other cereal pests, particularly gout fly, *C. pumilionis*. There was a significant reduction in numbers of gout fly larvae and an increased percentage of undamaged plants in the *T. stipitata* treatments.

Although gout fly are generally well controlled by parasitoids (Oakley & Young, 2000) their peak activity tends to coincide with the application of BYDV aphid pesticide treatments which may adversely affect these parasitoids and contribute to the increasing prevalence of this pest. Thus *T. stipitata* shows considerable promise as a generic treatment or the control of cereal pests.

This study has demonstrated the potential for the development of novel cereal pest control strategies based on the use of semiochemicals to manipulate both the pests and their associated beneficial insects. However, for robust control to be achieved, it is envisaged that such approaches will require semiochemical treatments to be combined into one overall strategy.

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**Clothianidin - a new chloronicotinyl seed treatment for use on sugar beet and cereals:  
Field trial experiences from Northern Europe**

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**ABSTRACT**

Results are presented from field trials on sugar beet and cereals, carried out across northern Europe between 1996 and 2001 to test the new active ingredient, clothianidin, for use as a seed treatment. Across all crops tested, clothianidin demonstrated a high level of plant compatibility and provided substantial protection against soil pests and in particular virus transmitting aphids. Results generally indicated that a lower rate of clothianidin could be used to achieve at least equivalent efficacy to the established chloronicotinyl, imidacloprid. Yield benefits in relation to pest and virus control are given for all crops. Also presented from France are indications of substantial yield benefits to be gained from the control of leafhoppers in winter wheat from this seed treatment.

**INTRODUCTION**

Clothianidin is the ISO common name for a new active ingredient, (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine, which belongs to the chloronicotinyl or neonicotinoid class of chemicals (Ohkawara *et al.*, 2002). It was initially discovered by Takeda Chemical Ind. Ltd and is currently being developed for world-wide use by Bayer as seed treatments in maize, cereals, sugar beet, oilseed rape and sunflower. Takeda are also developing it as foliar and soil treatments for top fruit, maize, potatoes, rice, vegetables, turf, tobacco and ornamentals.

Like the related and commercially available active ingredient, imidacloprid, it is effective against a wide range of sucking and biting pests, including many of those attacking sugar beet and cereals. By virtue of its systemic nature it is ideally suited for use as a seed treatment to protect against such pests.

The data presented is extracted from a large programme of work in each crop, carried out during the development of this new active ingredient as a seed treatment. Results are generally representative of northern Europe, as they have been collated from trials in Belgium, France, Germany, Holland, Sweden and the United Kingdom. Most pest problems were common throughout the locations tested, although in some countries results were obtained for less common pests, such as leafhoppers on winter wheat in France.

**MATERIALS AND METHODS**

Clothianidin was tested as a 600 FS formulation on sugar beet seed and as a 250 FS formulation in cereals. In sugar beet, treatments were applied by commercial seed treating companies (SUETT in continental Europe and Germain's UK Ltd in the U.K.) using their

standard procedures. In cereals, application was carried out by Bayer personnel using standard procedures and equipment that simulate the commercial process.

Trials were usually of a randomised block design with three to four replicates, although replicate number was increased to as much as six where pest distribution was more variable e.g. wireworms. Plot sizes ranged from 10-100 m<sup>2</sup> in cereals but were typically about 45 m<sup>2</sup> and in sugar beet the range was 15-48 m<sup>2</sup>, usually as four rows 10-15 m long. Plot sizes generally reflected the trial objective, tending to be larger for evaluation of virus control or crop safety and when yield was to be recorded.

Both crop safety and efficacy against crop establishment pests were evaluated by counting the number of plants in a given length of row e.g. 4-16 m per plot. Pest pressure was assessed directly by counting their numbers on a given sample size e.g. 25-100 plants/plot, or indirectly in terms of plant damage. Virus infection was assessed, usually on a whole plot basis, by visually estimating the percentage area infected. Furthermore, effects on the quantity of harvestable produce (and quality for sugar beet) were also measured at the end of the season, particularly where virus control or crop safety were being evaluated.

## RESULTS

### Sugar beet

#### Crop safety

In trials across 18 sugar beet cultivars no adverse effects were recorded on crop establishment, yield or quality even at double rates of use. Additionally, clothianidin has shown very good compatibility with both fungicidal seed treatments and herbicides.

Pests causing direct crop damage – soil pest complex and leaf miners

In the following tables, results are presented against a range of pests, individual species being summarised from those trials where they were the only or principal pest identified.

Table 1. Effect of seed treatments on sugar beet establishment in the presence of soil pests (% relative crop stand compared to the untreated control)

Treatment rate g a.i. / unit seed	% relative crop stands					
	<i>Agriotes</i> spp Crop GS 12-16		Soil pest complex* Crop GS 12-19		<i>Tipula paludosa</i> Crop GS 12	
	[Plants /m row]	(Number of trials)	[Plants /m row]	(Number of trials)	[Plants /m row]	(Number of trials)
Untreated control	[2.4]	(6)	[4.4]	(7)	[6.4]	(4)
Clothianidin 60	496.5	(6)	119.1	(7)	127.8	(4)
Imidacloprid 90	488.7	(6)	122.7	(7)	115.3	(4)

\*Results from sites where species of soil pests were mixed (springtails, *Onychiurus* spp.; millipedes, *Blaniulus guttulatus*; pygmy mangold beetle, *Atomaria lineatus* and symphylids, *Scutigera* spp).



Table 2. Effect of seed treatments against the direct feeding of pests on the hypocotyl and foliage of sugar beet (% reduction in the number of damaged plants cf. untreated)

Treatment rate g a.i./ unit seed	% reduction in damage			
	<i>Atomaria lineatus</i> Crop GS 14-18		<i>Pegomya hyoscyami</i> Crop GS 15-39	
	[% plants damaged]	(Number of trials)	[% plants damaged]	(Number of trials)
Untreated control	[24.9]	(5)	[52.5]	(11)
Clothianidin 45	83.7	(5)	*	*
Clothianidin 60	89.3	(5)	90.6	(11)
Clothianidin 90	95.5	(5)	*	*
Imidacloprid 90	93.1	(5)	72.4	(11)

\* Insufficient data points at this rate

Against the range of soil pests shown, a rate of 60 g clothianidin was found to be comparable to the highest rate of imidacloprid used in the commercial standard and was more effective against mangold fly, *P. hyoscyami*.

#### Aphids and Virus Yellows

Re-enforcing the strong effect against foliar pests are results on *Aphis fabae* and the main virus vector, *Myzus persicae*, with related effects on disease control and yield (Tables 3-4).

Table 3. Effects of seed treatments against aphids and virus yellows in sugar beet (% reduction in aphid numbers and virus symptoms in comparison with untreated)

Treatment rate g a.i. / unit seed	% reduction in aphids or virus yellows					
	<i>Myzus persicae</i> GS 14-19		<i>Aphis fabae</i> GS 16-42		Virus yellows 118-207 days after drilling	
	[Aphids /plant]	(Number of trials)	[Aphids /plant]	(Number of trials)	[% area]	(Number of trials)
Untreated control	[4.3]	(10)	[19.7]	(15)	[26.0]	(14)
Clothianidin 30-45	94.0	(10)	74.9	(14)	69.1	(14)
Clothianidin 60	96.8	(10)	86.6	(15)	77.7	(14)
Clothianidin 90	98.5	(10)	88.9	(14)	80.7	(14)
Imidacloprid 90	93.6	(10)	90.8	(15)	74.5	(14)

In terms of crop harvest, there was a general trend for both active ingredients to increase sugar yield, particularly in virus infected trials (Table 4). Analysis of amino-nitrogen at eleven sites also indicated a tendency for impurities to be reduced, again particularly when virus was controlled (data not presented) but statistical significance was only achieved on one occasion, therefore further work would be needed to confirm this effect.

Table 4. Effect of seed treatments on the yield of sugar from sugar beet, in the presence or absence of virus yellows (% relative yield in comparison with the untreated control)

Treatment rate g a.i. / unit seed	% relative sugar yield			
	Healthy No virus infection		Virus yellows 11-73 % infection	
	[t/ha]	(Significant/total trial results**)	[t/ha]	(Significant/total trial results**)
Untreated control	[11.4]	(2/4)	[10.2]	(5/7)
Clothianidin 45	*	*	114.5	(2/4)
Clothianidin 60	105.8	(2/4)	111.7	(5/7)
Clothianidin 90	105.8	(2/4)	116.0	(4/6)
Imidacloprid 90	103.0	(2/4)	113.6	(5/7)

\*rate not in this trials series.\*\*Significance ( $P < 0.05$ ) of treated compared to untreated yields. No statistically significant differences between imidacloprid and clothianidin, except clothianidin better in one 'healthy' trial.

## Cereals

### Crop safety

In trials across 30 cereal cultivars no adverse effects were recorded on crop establishment or yield even at double rates of use. Additionally, clothianidin has shown good compatibility with fungicidal seed treatments.

### Wireworms

Data on crop establishment and damage to emerged plants show clear benefits against wireworms, *Agriotes* spp. At sites where severe attacks had occurred protection against this pest translated into significant increases in grain yield (Table 5).

Table 5. Effects of seed treatments on crop establishment, feeding damage and yield in barley and wheat, at wireworm infested sites (% change in values cf. untreated)

Treatment rate g a.i./100 kg seed	% relative crop stand Crop GS 10-14		% damage reduction Crop GS 11-31		% relative yield	
	[Plants/m <sup>2</sup> ]	(Number of trials)	[% plants damaged]	(Number of trials)	[t/ha]	(Number of trials)
Untreated control	[153.5]	(11)	[29.1]	(13)	[1.9]	(3)
Clothianidin 35	117.4	(5)	52.7	(6)	149.0	(2)
Clothianidin 50	110.0	(11)	51.8	(12)	162.0	(3)
Clothianidin 70	113.6	(10)	61.3	(12)	175.7	(3)
Imidacloprid 70	106.7	(11)	44.8	(12)	125.3	(3)

Although not all data is orthogonal, there is a consistent trend across all rates and parameters that clothianidin gave at least comparable protection against wireworms to imidacloprid at 70

g a.i./100 kg seed (the highest European rate). The yield results taken from severely attacked sites confirm this useful efficacy but also show that under such conditions additional control measures will be necessary to ensure a good crop yield.

#### Aphids/BYDV

*Rhopalosiphum padi* and *Sitobion avenae* are the principal aphid species responsible for the transmission of BYDV to autumn sown crops of barley and wheat. Aphid results (Table 6) represent the combined data obtained against these species on both crops. Yield data is also combined for both crops, although BYDV results are for barley alone as this crop provided the greatest and most reliable symptom expression. There were no major differences inherent in the data to suggest that the relative performance of clothianidin was markedly influenced by crop or aphid species, therefore the combined data provides a more reliable picture of overall performance.

Table 6. Control of aphids (*R. padi*; *S. avenae*) and BYDV in relation to grain yield in winter barley and winter wheat.

Treatment rate g a.i. / 100 kg seed	% reduction in aphids, BYDV or % relative yield					
	Aphids		BYDV		Yield	
	Crop GS 12-25 [% plants infested]	(Number of trials)	Crop GS 25-67 [% area infected]	(Number of trials)	[t/ha]	(Number of trials)
Untreated control	[28.7]	(22)	[30.3]	(26)	[6.4]	(13)
Clothianidin 35-37.5	97.6	(13)	95.9	(23)	141.0	(11)
Clothianidin 50	99.7	(14)	95.9	(23)	149.5	(10)
Clothianidin 70	99.2	(21)	97.9	(11)	*	*
Imidacloprid 35	88.3	(9)	88.9	(14)	131.7	(6)
Imidacloprid 70	98.3	(22)	95.5	(24)	139.2	(12)

\* Insufficient data points at this rate

The results presented in Table 6 show that a generally high level of efficacy was achieved by both chloronicotinyl active ingredients and that across all parameters clothianidin was at least as effective as the standard, imidacloprid, even when used at lower rates.

#### Leafhoppers

Results from four French trials conducted over two years, demonstrate that clothianidin can provide very useful benefits from suppressing the activity of the leafhopper *Psammotettix alienus*, which is responsible for the transmission of wheat dwarf and Russian mosaic viruses (Table 7). The data presented shows that not only was a significant effect recorded against *P. alienus* during early spring (March/April) but that this was of sufficient magnitude to translate into significant increases in grain at harvest. In terms of reduced damage to plants, clothianidin did not appear to be quite as effective as imidacloprid at the equivalent a.i. rate, although yield benefits were comparable.

Table 7. Suppression of leafhopper (*P. alienus*) in winter wheat (% change in damage and yield in comparison with the untreated control)

Treatment rate g a.i. / 100 kg seed	% damage reduction, GS 31-45		% relative yield	
	[% plants damaged]	(Number of trials)	[t/ha]	(Number of trials)
Untreated control	[18.0]	(4)	[6.6]	(3)
Clothianidin 50	52.9	(4)	122.0	(3)
Clothianidin 70	52.5	(4)	121.1	(3)
Imidacloprid 70	68.4	(4)	125.5	(3)

## DISCUSSION

As a seed treatment for sugar beet and cereals, clothianidin has demonstrated high levels of effect against a range of pests. It has shown particular strengths against foliar pests and importantly viruliferous aphids, where it clearly raises the standard of control available from this form of application. In all crops, clothianidin delivered at least equivalent virus control and yield benefits to the chemically related commercial standard, imidacloprid, even when used at lower rates of application.

Useful effects against other arthropod pests have also been demonstrated. Protection against the spectrum of soil pests in sugar beet was generally comparable to that achieved by imidacloprid and superior to its activity against mangold fly, *P. hyoscyami*. In cereals clothianidin also protected against wireworms and leafhoppers. However, in severe wireworm situations, additional control measures would be necessary to ensure a commercially acceptable result. The data obtained against leaf hoppers suggests that this pest may be more damaging than originally thought and could be a practical consideration when planning chemical control strategies.

In conclusion, the chloronicotinyl clothianidin builds on the established success of this chemical group for seed treatment, to raise the standard of protection of yield and quality in both sugar beet and cereal crops.

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