AUGMENTATION BIOLOGICAL CONTROL USING THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA FELTIAE* AGAINST THE SOUTH AMERICAN LEAFMINER *LIRIOMYZA HUIDOBRENSIS*

J. Head and K.F.A. Walters
Central Science Laboratory, DEFRA, Sand Hutton, York, United Kingdom

INTRODUCTION

Since its introduction into the United Kingdom in 1989, the South American leafminer, *Liriomyza huidobrensis* (Blanchard), has caused outbreaks annually in protected crops. In response, statutory measures are routinely taken to eradicate the pest from propagation premises and to prevent movement of the pest to other commercial sites (Cheek *et al.*, 1993; Cannon *et al.*, 1997).

Routine measures used to control the larval stages of non-statutory leafminer species in the United Kingdom rely largely on either the use of the braconid parasitoid *Dacnusa sibirica* Telenga and the eulophid wasp *Diglyphus isaea* Walker as, for example, in tomato crops, or on chemical insecticides for control in leafy salad and ornamental plants (Garthwaite and Thomas, 2001). The availability of effective chemical insecticides for use against leafminers is severely limited, and the introduction of parasitoids alone to control leafminer populations requires repetitive releases of large numbers (Sher *et al.*, 2000), which would be prohibitively expensive. Therefore to reliably achieve eradication a new approach was required. A non-chemical control method using the entomopathogenic nematode *Steinernema feltiae* (Filipjev) as a foliar treatment against larval instars was developed (Williams and Macdonald, 1994; Williams and Walters, 1995, 2000). However, before nematodes could be integrated into pest management programs, information on their compatibility with chemical insecticides was required. Earlier studies addressed the consequences of direct exposure to solutions of insecticides on the behavior and infectivity of selected entomopathogenic nematode species; however, combinations of insecticides and nematode species available for use in the United Kingdom were not included. Additionally, the effect of dry pesticide residue on subsequent nematode applications had not previously been investigated.

This paper describes the investigations undertaken to establish the compatibility of *S. feltiae* with selected chemical insecticides by assessing first the effect of direct exposure of *S. feltiae* infective juveniles (IJs) for 24 hours to insecticide and, second, the application of IJs to foliage-bearing insecticide residues. We also present results of a glasshouse trial with *S. feltiae* conducted to validate some of our laboratory results.

MATERIALS AND METHODS

Direct Exposure

Infective juveniles of *S. feltiae* (as Nemasys®, BeckerUnderwood U.K. Ltd) were suspended in solutions of insecticides (abamectin as Dynamec 50 ml/100 liters of water, deltamethrin as Decis 70 ml/100 litres of water, dimethoate as Danadim 85 ml/100 litres of water, heptenophos as Hostaquick 75 ml/100 litres of water and trichlorfon as Dipterex 80 1.5 kg/1000 litres of water) prepared at the recommended concentration for use in protected crops in the United Kingdom. The suspensions were incubated at 20° ± 1°C in the dark for 24 hours. After this period 1 ml of each of the nematode suspensions were removed and washed to separate the nematodes from the chemicals using the method of Rovesti and Deseo (1990). The infectivity of the washed nematodes was determined by a standard bioassay

method using *Galleria mellonella* (L.) (Fan and Hominick, 1991), and results were compared against the infectivity of the control of *S. feltiae* incubated for 24 hours in water.

Exposure of *S. feltiae* to Insecticide Residues

Lettuce plants (*Lactuca sativa* L.) infested with *L. huidobrensis* at the second to third instar larval stages, as determined by the size of the mouth hook structures using the measurements reported by Head *et al*. (2002), were divided into four treatment groups. The plants were incubated in an environmental chamber at 20 ± 1°C, 12:12 hours L:D regime, and 65% r.h. Each treatment group received a combination of two sequential treatments.

The first treatment consisted of either an insecticide applied at the recommended dose rate for application to greenhouse-grown lettuce in the United Kingdom or a water control, and in each case plants were sprayed to run-off. The plants were returned to the environmental chamber until the second treatment was applied 24 hours later. This consisted of either a suspension of 10,000 *S. feltiae*/ml, with 0.02% of a nonionic wetting agent Agral (a.i., alkyl phenol ethylene oxide) or a water (control), also sprayed to run-off. Following the second treatment, the plants were incubated at >85% r.h. for 12 hours in the dark.

Nematode efficacy was assessed by the numbers of live and dead (nematode infected) larvae, and pupae present five days after the first treatment (when the majority of live individuals had pupated) for each replicate in each treatment group. The survival of *L. huidobrensis* was expressed as the percentage of live larvae and pupae in each treatment group compared with the control.

Glasshouse Trial

A 120 m^2 plot of Chinese brassica (*Brassica rapa* var. *chinensis*) plants within a commercial production glasshouse that was heavily infested with *L. huidobrensis* was selected for a trial of the combined use of *S. feltiae* and insecticide. The plot was divided into a control and an experimental area. Deltamethrin (as Decis®) was applied to both areas seven days before a foliar application of *S. feltiae* (at 5,000 *S. feltiae*/ml, containing 0.02% Agral v/v) to the experimental area. Immediately before and 48 hours following *S. feltiae* application, plants were sampled from both areas and samples were incubated to monitor the numbers of larvae surviving to pupation. The mean number of pupae per plant was used to estimate the effectiveness of the *S. feltiae* application. Glasshouse temperatures were recorded during the 48 hours following nematode application to ensure they remained within the effective range for nematode activity.

RESULTS

Direct Exposure

There was a significant difference in the infectivity of *S. feltiae* following direct exposure to the different active ingredients. Exposure to trichlorfon resulted in an infectivity level of 38.8%, not significantly different from the 51.0% obtained for the controls ($F=1.28$. d.f.=1,18, $P>0.05$). An acceptable level of nematode infectivity (27.3%) was also obtained for dimethoate, but it was significantly lower than the control ($F=10.43$, d.f.=1, 18, $P<0.05$). Nematodes exposed to abamectin, deltamethrin, or heptenophos were found to have very low infectivity, i.e., 0.1% (abamectin), 0.6% (deltamethrin) and 0.6% (heptenophos), values which are too low to be of practical value.

Exposure of *S. feltiae* to Insecticide Residues

The percentage survival of *L. huidobrensis* treated with insecticide alone was very high and attributable to the high levels of resistance exhibited by this culture (Fig. 1, Treatment group b – "insecticide and water"). Abamectin was the only insecticide that caused a reduction in survival of *L. huidobrensis* in the absence of the nematodes, with an 80% survival recorded in tests using this chemical. Controls with water alone (Fig. 1, treatment group a – "water alone") caused negligible mortality during the experimental period. The high post-treatment survival of leafminer larvae enabled investigation of the effect of chemical residues on nematode activity.

The nematodes achieved significant levels of control of *L. huidobrensis* (Fig. 1, Treatment group d – "water and *S. feltiae*"). Comparing the water control with applications of *S. feltiae* that followed an insecticide (Fig. 1, Treatment groups c – "insecticide and *S. feltiae"*), a significant difference ($P < 0.001$) was recorded in all cases. After allowing for the variability between replicates, there were no significant differences between the survival of *L. huidobrensis* when treated with *S. feltiae* only (treatment d) or in combination with insecticides (treatment groups c – "insecticide and *S. feltiae*"; all $P > 0.1$, 1 d.f., largest $c^2 = 1.60$ for abamectin).

In addition to larval mortality, in a few cases application of *S. feltiae* to late instar larvae nearing pupation resulted in the formation of nonviable pupae. As only larval mortality was scored, the results in Fig. 1 represent the minimum efficacy of the nematode treatments.

Glasshouse Trial

Before application of *S. feltiae*, means of 5.9 and 5.1 pupae per plant were recorded from the experimental (insecticide and *S. feltiae*) and control (insecticide only) plots, respectively. Following the application of *S. feltiae* to the experimental plot the mean number of pupae was reduced by over 88% compared with the number of pupae that emerged from the control plot (Table 1). The mean temperature over the 48 hour period following the nematode application was 19.7 °C (range 17.4 - 24.1 °C).

DISCUSSION

The work identified two approaches for the successful integration of chemical insecticides with entomopathogenic nematodes. A limited range of insecticides (trichlorfon and dimethoate) can be applied simultaneously with *S. feltiae*. Alternatively, high levels of control of leafminer larvae can be achieved by the application of *S. feltiae* to vegetable foliage previously treated with any of the five insecticides used in this study. Integrated Pest Management approaches may require sequential rather than simultaneous applications of chemical insecticides and entomopathogenic nematodes. The results of the glasshouse trial demonstrate the efficacy of the nematodes when used within an existing treatment regime operated by the grower. Sequential treatments offer a greater flexibility in timing applications of the different control agents, many of which are known to cause differential mortality to the various life stages of this pest (Williams and Walters, 1994). Thus targeting of a particular life stage with the most appropriate control measure remains a viable option.

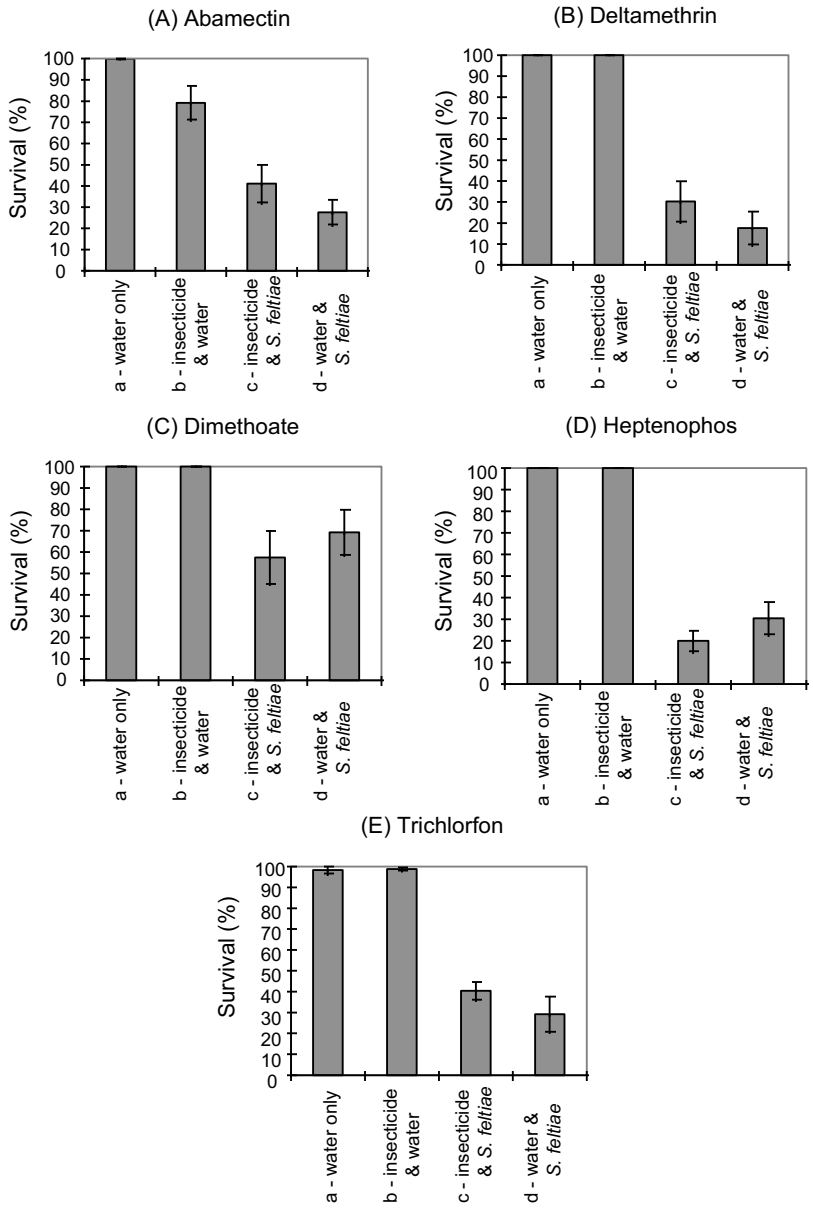

Figure 1. Effect of dry residue of five insecticides on the efficacy of *S. feltiae* (10,000 IJ/ml) to control *L. huidobrensis* larvae. Leafminer larval survival (%) within one of four treatment groups: (a) water alone, (b) pesticide and water, (c) pesticide and *S. feltiae*, and (d) water and *S. feltiae*.

Table 1. Mean number of pupae collected from plants sampled from the insecticide only (control plot) and the experimental (insecticide and *S. feltiae*) treated plot before and after the nematode application.

	Pre-application of *S. feltiae*		Post-application of *S. feltiae*		
Treatment	No. plants	Mean no. pupae per plant (± s.e.)	No. plants	Mean no. pupae per plant (± s.e.)	Reduction (%)
Insecticide and *S. feltiae*	17	5.90 (± 1.15)	16	0.41 (± 0.17)	88.98
Insecticide only	17	5.12 (± 1.27)	16	5.29 (± 0.99)	0.00

REFERENCES

Cannon, R. J. C., J. Head, S. Cheek, and P. W. Bartlett. 1997. The pattern and development of outbreaks of the South American leaf miner, *Liriomyza huidobrensis*, in the United Kingdom, pp 443-450. *In* Anon. *Fourth International Conference on Pests in Agriculture*, A.N.P.P., Montpellier, France.

Cheek, S., O. C. Macdonald, and P. W. Bartlett. 1993. Statutory action against *Liriomyza huidobrensis* in the United Kingdom, pp. 79-86. *In* Anon. *Proceedings of Liriomyza conference on leaf mining flies in cultivated plants,* Montpellier, France.

Fan, X. and W. M. Hominick. 1991. Efficiency of the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae and Heterorhabditidae) from soil and sand. *Revue de Nematologie* 14: 381-387.

Garthwaite, D. G. and M. R. Thomas. 2001. Pesticide Usage Survey Report 164: Protected Crops (Edible and Ornamental) in Great Britain in 1999. Department of Environment, Food and Rural Affairs, London.

Head, J., K. F. A. Walters, and S. Langton. 2002. Utilisation of morphological features in life table studies of *Liriomyza huidobrensis* developing in lettuce. *Journal of Applied Entomology* 126: 349-354.

Rovesti, L. and K. V. Deseo. 1990. Compatibility of chemical pesticides with the entomopathogenic nematodes, *Steinernema carpocapsae* Weiser and *S. feltiae* Filipjev (Nematoda: Steinernematidae). *Nematology* 36: 237-245.

Sher, R. B., M. P. Parrella, and H. K. Kaya. 2000. Biological control of the leafminer *Liriomyza trifolii* (Burgess): implications for intraguild predation between *Diglyphus begini* Ashmead and *Steinernema carpocapsae* (Weiser). *Biological Control* 17: 155-163.

Williams, E. C. and O. C. Macdonald. 1995. Critical factors required by the nematode *Steinernema feltiae* for the control of the leafminers *Liriomyza huidobrensis*, *Liriomyza bryoniae* and *Chromatomyia syngenesiae*. *Annals of Applied Biology* 127: 329-341.

Williams, E. C. and K. F. A. Walters. 1994. Nematode control of leafminers: Efficacy, timing and temperature, pp. 1079-1084. *Brighton Crop Protection Conference–Pests and Diseases*. The British Crop Protection Council, Farnham, Surrey, United Kingdom.

Williams, E. C. and K. F. A. Walters. 2000. Foliar application of the entomopathogenic nematode, *Steinernema feltiae* against leafminers on vegetables. *Biocontrol Science and Technology* 10: 61-70.