Project PC/22

THE SOUTH AMERICAN LEAFMINER *LIRIOMYZA HUIDOBRENSIS*

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THE SOUTH AMERICAN LEAFMINER LIRIOMYZA HUIDOBRENSIS

Project co-ordinator: John Overvoorde

Project leader: Neil Helyer

Location: Horticulture Research International, Littlehampton, West Sussex, BN17 6LP, UK

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Working team: Neil Helyer, Graeme Gill and the HRI nursery staff for producing the plant material

ABSTRACT

The biology and behaviour of this non-indigenous leafminer was investigated on a range of plants, as it is subject to statutory control it had to be worked with under strict quarantine conditions. The pest attacks a wide range of horticultural crops including many edible and ornamental plants. Our studies have shown that broad bean and cucumber are the preferred host plants and that over-wintering pupae are sensitive to low humidity conditions. Also, the average female fly produces over 6,500 oviposition marks, 163 eggs and can live for more than 23 days. Males however, cannot feed in the absence of a female and generally live no more than 4 days. Biological control of pupae in soil was tested and the insect parasitic nematode *Steinernema feltiae* proved to be the most effective agent. Twenty-six pesticides were evaluated against the leafminer parasites *Dacnusa sibirica* and *Diglyphus isaea*, which can be used to control this and other leafminer species.

INTRODUCTION

The South American leafminer *Liriomyza huidobrensis* is a polyphagous pest of many plant species, the fly attacks most horticultural crops including, chrysanthemum, celery, cucumber, lettuce, tomato, brassicas and several other edible and ornamental crops. The adult fly is almost identical to our native tomato leafminer *L. bryoniae*; 2.5 mm long, black with red eyes on a yellow head and having a noticeable yellow spot on

the thorax between the wings. Damage is principally by mining through the leaf. Mines can appear erractially on the upper side of the leaf, disappear for a distance and reappear further on, the mines occasionally run along main and secondary veins. Since arriving in Europe in 1989 it has been found to be resistant to many pesticides but able to be controlled biologically by the parasites *Dacnusa sibirica* and *Diglyphus isaea*. The work conducted at HRI has examined the biology and behaviour of this pest, potential biological control of the pupal stage the effects of pesticides on its parasites.

METHODS

A culture of *L. huidobrensis* was maintained in a quarantine unit on a range of plants, including; broad bean (cv Colossal), chrysanthemum (cv Pink Gin) cucumber (cv Mildana) and tomato (cv Money maker). Throughout most of the trial all the species of plants were infested together, thus giving a choice of host plant to prevent the insect becoming restricted to one type of plant. Adult flies were maintained in a cage fitted with sleeved gloves to facilitate handling without loss of flies. Due to the high number of adults kept in the cage, infesting of clean plant material could be done within 5-6 hours, thus keeping a series of synchronous cultures ready for experimental use. Once infested the plants were vigorously shaken to remove the adults and then transferred to further cages for development to the required stage for testing. Those leafminers reaching pupation were collected and returned to the adult infesting cage for later emergence. During the trials (see trial 1) it became obvious that low humidity adversely affected adult emergence from the puparia, to counter this puparium awaiting emergence were placed in Petri dishes over wet capillary matting.

Trial 1. Effects of short day lengths on *Liriomyza huidobrensis*

A separate culture was set up during the months of September to February to investigate the effects of winter daylengths on the pupation of *L. huidobrensis*. These insects were reared in large (90 x 60 x 50 cm) perspex cages close to north facing windows, thus receiving the short hours of winter daylight but no direct sunshine. Pupae were collected into plastic Petri dishes and maintained next to the rearing cages, the colour and number of pupae emerging were recorded from each culture throughout these months (Table 1).

Table 1. Survival rates of different colour pupae under natural daylength/daylight conditions

Date collected	Dark	Number emerging	Medium	Number emerging	Light	Number emerging
30 September	181	106	179	76	32	13
28 October	4	4	21	7	4	2
7 November	97	43	124	51	18	5
18 November	64	42	89	36	9	5
22 November	36	15	31	16	3	1
2 December	65	35	64	34	6	3
9 December	106	77	120	50	4	0
16 December	66	31	50	11	10	2
20 December	35	20	40	20	8	5
24 December	29	22	79	43	20	6
30 December	54	27	50	24	5	2
9 January	37	22	11	4	1	0
13 January	69	48	103	53	34	11
23 January	19	7	45	5	10	0
27 January	43	24	49	22	34	8
3 February	33	24	49	25	20	3
13 February	90	52	66	39	8	2
20 February	118	65	166	66	37	3
21 February	74	47	55	21	25	6
TOTALS	1220	711	1391	603	288	77
% emergence	58.2%		43.3%		26.7%	

Trial 2. Host plant preference

Host plant preference was tested on broad bean, chrysanthemum, cucumber and tomato (cv's as above). An aluminium test cage (Helyer, 1991) was set up with leaf sections (8 cm^2) mounted on agar poured over a glass plate. The sections of leaf were placed on agar just at the point of setting so as not to scald the leaves. The leaves were placed abaxial surface uppermost on one sheet of glass and adaxial surface uppermost

on the other. Thus when the cage was assembled the abaxial leaf surface was positioned above the adaxial surface presenting the leaves to the flies as they would be on the living plant. Individual cages were used for each host plant tested (n = 4). Assembled cages were maintained in an illuminated and air conditioned growth cabinet (Fisons Fitotron 600) for the duration of the trial; temperature 22°C, 16 h daylength and humidity at 60-80%.

Two freshly emerged (C. 12 h) female flies were placed in each of the test cages and the leaves within changed every 2 days over a 10 day period. The number of oviposition/feeding marks were counted on each leaf and then all leaves were removed from the agar and gently boiled in a lactophenol/acid fuchsin stain solution (Parrella and Robb, 1982) to facilitate egg counts (Table 2). In a separate test, leaves from all four species of plant were placed in the same cage together (Table 3) so as to determine the preferred host plant.

Table 2. Mean number of oviposition marks and eggs laid per female, per day over a 10 day period when given no choice of host plant

Plant	Oviposition marks			Eggs laid		
	Upper	Under	Total	Upper	Under	Total
Broad bean	181	30	211	9.45	2.75	12.2
Chrysanthemum	43	129	172	0.25	1	1.25
Cucumber	140	156	296	1.75	1	2.75
Tomato	145	74	219	0.8	0.9	1.7

Table 3. Mean number of oviposition marks and eggs laid per female, per day over a 10 day period when given a choice of host plant, i.e. leaves of each host plant presented simultaneously

Plant	Oviposition marks			Eggs laid		
	Upper	Under	Total	Upper	Under	Total
Broad bean	59.5	57.5	117	1.275	1.875	3.15
Chrysanthemum	1	0.11	1.11	0.05	0.025	0.075
Cucumber	37.5	47.5	85	1.2	1.05	2.25
Tomato	6.5	4.5	11	0.3	0.15	0.45

Trial 3. Longevity and fecundity on chrysanthemum

Four flies (2 male and 2 female) were placed in each of 10 aluminium test cages. Chrysanthemum leaves (cv Pink Gin) were mounted on agar as above, with abaxial and adaxial surfaces exposed to the flies. Leaves were changed daily, when a death occurred the fly was sexed and removed but no replacement made. The number of oviposition marks were counted on each leaf and 5 replicates of abaxial and adaxial leaves stained in lactophenol/acid fuchsin solution to determine the number eggs per leaf.

Trial 4. Evaluation of pesticides for side-effects on the leafminer parasites *Dacnusa sibirica* and *Diglyphus isaea*

The parasites *Dacnusa sibirica* and *Diglyphus isaea* are important leafminer parasites on a range of edible and ornamental crops. Twenty six pesticides (9 insecticides, 11 fungicides and 6 acaricides) were evaluated against both parasites in the adult stage. Adult parasites were supplied throughout the trial by Koppert UK as "Minusa" (*Dacnusa sibirica*) and "Miglyphus" (*Diglyphus isaea*) in polythene bottles each containing 250 flies. A source of food was provided in the lid of each bottle in the form of a felt pad soaked in honey:water solution. Adults delivered thus were comparable to those supplied to growers and could survive for over 1 week in the container.

The insects were anaesthetized with CO_2 and collected by aspirator into 7 ml plastic bijou bottles (15-20 per bottle), the lids of which were covered with a fine mesh. Leaf discs (22 mm diam.) were cut from pesticide-free chrysanthemum plants and placed, abaxial surface uppermost, on wet filter paper. These were then sprayed with 0.2 ml of 50:50 honey:water solution per replicate, through a Potter tower (Potter, 1952).

Pesticides were prepared at 10 x the manufacturers highest concentration and diluted to 1 X and 1/10 X in tap water. Pesticides (2 ml) were applied to the leaf discs for each replicate through a Potter tower. This dose was equivalent to 6.38 mg fluid per leaf disc (2.9 mg fluid cm^{-2}). Controls were similarly sprayed with tap water. Treated leaf discs were left to dry at 20°C for 30 minutes before the test cages (Ledieu, 1979) were assembled. The insects were once again anaesthetized with CO_2 by gassing through the meshed lid of the bijou bottle before release into the test cage. An assessment of initial handling mortality was taken some 30 minutes later, further mortality was assessed at 24 and 48 h after caging the parasites. Control mortalities were consistently between 0-6% and accounted for by the use of Abbott's correction formula (Abbott, 1925).

Trial 5. Biological control of *L. huidobrensis* pupae in soil compost

Most *Liriomyza* species of leafminer pupate externally from the leaf on which they fed as larvae. Some remain attached to either the upper or lower surface of the leaf while others fall to the ground, in doing so they may land on a polyethylene covered floor (most cucumber, sweet pepper and tomato crops) or onto soil (chrysanthemum, lettuce and other ornamental crops). Those landing on polyethylene should be controlled by an

application sticky material such as 'Superstick' or 'Thripstick' which can remain active for several weeks after application (Helyer *et al.*, 1983). Larvae finding their way to a soil compost will generally bury themselves a centimetre or so below the surface and thus be protected until emergence as an adult.

Trials were conducted to determine the potential of a biological control for this pest during the pupal stage in compost. The compost used throughout the trials was a freshly autoclaved mixture of 50:50 by volume of loam and peat (Shamrock Irish, medium grade, Sphagnum peat). This mixture was chosen as a representative glasshouse soil as found in a typical chrysanthemum or lettuce house. Peat and loam were thoroughly mixed in a mechanical soil mixer before being bagged and autoclaved, fresh compost was used for each trial. Once autoclaved and cooled the moisture content of the compost was between 17 and 22%. Plastic propagator trays (160 x 220 x 50 mm) (Stewart Co. Ltd.) were filled with 1 litre of mixed compost to a depth of 40 mm. The trays were filled to leave a loose substrate with a proportion of organic material (peat) as a preferred pupation site for soil pupating insects (Varatharajan & Mohan Daniel, 1984), this was standard throughout the trials.

A galvanized steel (Weld-Mesh^{R}) grid made from 3.5 mm diameter wire, welded at each cross-over point, to make 25 mm x 75 mm spaces was folded at the edges to make legs. The wire grid was placed on the tray with the legs pushed into the compost to leave a 1 cm gap between the grid and the compost. Broad bean leaves bearing a known number of late instar leafminer larvae were placed over the wire grid and after 3-4 days the leaves and any remaining larvae were removed. Thus leaving a known number of insects in the compost.

Three compost inhabiting biological control agents were evaluated; the fungal pathogen *Metarhizium anisopliae*, the insect parasitic nematode *Steinernema feltiae* and the predatory mite *Hypoaspis miles*. Both the nematode and predatory mite were introduced to the compost just before the leafminer larvae. This allowed the larvae to be attacked as they entered the compost to pupate. However, the fungal pathogen was applied 1 week before the leafminer larvae to enable it to germinate and spread through the compost. Controls were treated with water.

After treatment, the propagator lids were sealed to the trays with 50 mm wide masking tape which trapped any insects escaping under the edge of the cage. A paper clip was inserted, centre top of the lid and a 5 cm square of yellow plastic sticky trap hooked on to it. The complete propagators were maintained at 22°C and 16 : 8, light : dark regime for 4 weeks after treatment to allow total emergence of surviving adults. Five such replicates were set up for each treatment.

RESULTS AND DISCUSSION

Trial 1. Effects of short day lengths on *Liriomyza huidobrensis*

Fewer light and more dark coloured pupae were formed during the period of this trial (Table 1). This conforms closely with previous work on *Liriomyza bryoniae* in which dark pupae took longer to begin emerging but continued over a longer period of time. In the present study dark pupae were found to be more robust and better able to withstand harsher conditions than the light coloured pupae. This is important for survival during the winter months and indicates that pupae could remain viable between crops. Light coloured pupae however, were more susceptible to low humidity (average humidity in the quarantine unit was 40% Rh) they may therefore be triggered to emerge more quickly, as happens during the summer months.

Trial 2. Host plant preference

Adult leafminers feed on the plant sap produced when a female fly inserts her ovipositor into the leaf surface seeking a suitable site to insert an egg. These oviposition marks soon become noticeable as pale white spots visible from the upper leaf surface. Male flies (no ovipositor) cannot feed in the absence of a female fly and generally die within 4 days of emerging from the puparium, it was also observed that during the first 3 days of the adults life much feeding takes place but no eggs are laid.

Four species of plant were offered to the flies to determine the preferred host plant. The results of this trial are presented in Table 2, although cucumber was preferred for feeding, broad bean was preferred for egg laying. In the second host plant preference test, all species of plant were presented to the adults simultaneously thus giving a choice for feeding and oviposition. In this test (Table 3) the order of preference was; broad bean > cucumber > tomato > chrysanthemum. No preference was noticeable in either abaxial or adaxial leaf surfaces of any particular plant. This indicates that pesticide control aimed at the adult fly must be directed at both leaf surfaces to ensure adequate contact with the fly. This also suggests that cucumber and broad bean should make useful trap/monitor plants for early notification of *L. huidobrensis* outbreaks.

Trial 3. Longevity and fecundity on chrysanthemum

No eggs were laid during the first 3 days of the adult females life but, as in trial 2, many oviposition marks were made purely for feeding. Eggs, once production had begun were produced continuously for approximately 20 days. The average number of oviposition marks made by a female was 6,509 at a ratio of 40:1, oviposition marks : eggs and an average of 163.6 eggs per female during her life. Average lifespan was 23.5 days for females and 4 for males with a maximum of 31 and 9 days respectively. These results were from flies reared on chrysanthemum, so higher numbers of oviposition marks and eggs would be expected from a more favoured host plant.

Trial 4. Evaluation of pesticides for side-effects on the leafminer parasites *Dacnusa sibirica* and *Diglyphus isaea*

The results (Table 4) are expressed as one of four categories: 1 (Safe) = <30% mortality, 2 (Slightly harmful) = 30-79% mortality, 3 (Moderately harmful) = 80-99% mortality and 4 (Harmful) = >99% mortality. They are taken from the mean of the 4 replicates at 1 X pesticide concentration at 48 h after caging. The categories correspond with those of the International Organisation for Biological Control working group 'Pesticides and Beneficial Organisms' initial laboratory screening tests. The insecticides heptenophos, nicotine and trichlorphon were all more harmful to *D. isaea* than *D. sibirica*, however the opposite occurs with pirimicarb and pyrethrins plus resmethrin. Most of the fungicides with the exception of pyrazophos were safe to both species, similarly with the acaricides (except avermectin). However, the more specific insect growth regulating compounds (diflubenzuron and teflubenzuron) show absolute safety to these adult parasites at up to 10 X the highest recommended concentration.

Trial 5. Biological control of *L. huidobrensis* pupae in soil compost

The number of adult flies surviving the treatment and being trapped were counted 4 weeks after setting up the propagators. The nematode *Steinernema feltiae* proved to have the best potential with only 10.6% survival compared to 86.6% in the control. However, this is not good enough control for a statutory notifiable pest requiring complete eradication, but is a useful measure of pest reduction. The other treatments; *Metarhizium anisopliae* and *Hypoaspis miles* had 35% and 47.3% survival respectively with a mean survival of 77.4% in the controls.

Table 4. Side-effects of pesticides on *Diglyphus isaea* and *Dacnusa sibirica*

Pesticide	Trade name	Manufacturer	Rate of use mg a.i./l (@ 1x conc^{n})	*D. isaea*	*D. sibirica*
INSECTICIDES					
Bacillus thuringiensis	Thuricide 16,000 IUP/mg	Sandoz	$1.6x10^{7}$	1	1
diazinon	Diazinon 16% e.c.	DowElanco	160	4	4
diflubenzuron	Dimilin 25% w.p.	ICI	125	1	1
heptenophos	Hostaquick 55% e.c.	Hoechst	412.5	4	3
nicotine	XL-All Insecticide 7% e.c.	Synchemicals	1120	4	2
pirimicarb	Pirimor 50% w.p.	ICI	250	1	3
pyrethrins + resmethrin	Pynosect 30% e.c.	Mitchell	91+588	3	4
teflubenzuron	Nemolt 15% s.c.	Fargro	75	1	1
trichlorphon	Dipterex 80% w.p.	Bayer	1200	4	3
FUNGICIDES					
benomyl	Benlate 50% w.p.	Du Pont	500	1	1
bupirimate	Nimrod 25% e.c.	ICI	312.5	2	1
chlorothalonil	Repulse 50% s.c.	ICI	1100	1	1
dichlofluanid	Elvaron 50% w.p.	Bayer	500	1	2
fenarimol	Rubigan 12% s.c.	DowElanco	216	1	1
imazalil	Fungaflor 20% e.c.	Hortichem	100	1	1
propamocarb	Filex 72.2% e.c.	Fisons	1083	1	1
propiconazole	Tilt 25% e.c.	Ciba-Geigy	100	1	1
pyrazophos	Afugan 30% e.c.	Hoechst	150	3	4
sulphur	Thiovit 80% m.g.	PBI	1600	2	1
triforine	Saprol 19% e.c.	Promark	237.5	1	1
ACARICIDES					
avermectin	Dynamec 1.8% e.c.	MSD Agvet	4.5	3	2
dienochlor	Pentac 48% e.c.	DowElanco	312	1	1
fenbutatin oxide	Torque 50% w.p.	ICI	250	1	1
petroleum oil	Spraying oil 71% e.c.	Hortichem	7100	1	1
quinomethionate	Moristan 25% w.p.	Hortichem	125	1	1
tetradifon	Tedion 8% e.c.	Hortichem	120	1	1

CONCLUSIONS

While *L. huidobrensis* remains a notifiable pest strict measures must be taken to limit its spread and try to eradicate it; this inevitably means chemical control. Pesticides used against this pest effectively prevent the use of biological control for other pests. However, if *L. huidobrensis* becomes established there will be an immediate need for an integrated pest management strategy. This leafminer shows great capacity for reproduction and can cause excessive damage to several plants. It has been shown that broad bean and cucumber are very susceptible and that a possible area of weakness lies in the vulnerability of the pupae to conditions of low humidity. The results from this work indicate that certain pesticides are available to integrate with these parasites to control other pest and disease symptoms in a full IPM programme.

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