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Control of the Leafminer, *Liriomyza huidobrensis* in Potatoes Through IPM

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Table of Contents

Executive Summary 3

I. Research Objectives 4

II. Methods and Results 6

A. Survey of *Liriomyza huidobrensis* and parasitoids in Peru 6

B. Seasonal population fluctuations in Peru 12

C. Survey of *L. huidobrensis* and parasitoids in Israel 15

D. Mass rearing of *L. huidobrensis* and parasitoids in Peru 17

E. Effect of translaminar insecticide on *L. huidobrensis* larvae 20

III. Impact, Relevance, and Technology Transfer 29

IV. Project Activities/Outputs 31

A. Meetings 31

B. Training 32

Of CIP personnel 32

Of growers, university students, foreign visitors by CIP 32

C. Publications and patents 33

V. Project Productivity 34

VI. Future Work 35

VII. Literature Cited 36

VIII Appendix – publications 37

Executive Summary

The pea leafminer, *Liriomyza huidobrensis* (Blanchard), a native to South America, spread to Europe, Israel and Africa as a pest in which the adults are refractory to insecticides. Leafminers are highly polyphagous, attacking flower and vegetable crops in open fields and greenhouses. Because there are only a limited number of insecticides that are efficacious against the larvae, insecticide resistance problems, even in the larvae, could quickly become a reality.

The objectives of this project were to determine the array of natural enemies (parasitoids) and the extent of control each exerts on the leafminer; to evaluate the effect of larvicides, and to develop a strategy using parasitoid releases and minimal amounts of insecticides to control the leafminer. Surveys were conducted in all of the coastal departments of Peru on 23 crop plants for the leafminer and natural enemies present. Seventeen wasp species were collected and most were identified; the most promising were selected for mass rearing. Mass rearing techniques for the leafminer were developed; optimum temperatures and densities determined, and facilities were built. The leafminer and natural enemy production commenced.

Trials on the effects of larval insecticides (abamectin, cyromazine, spinosad, cartap and chlorfluazuron), botanical (*Sapindus saponaria*) and fungi (*Paecilomyces fumosoroseus*, *Beauveria bassiana*) on the leafminer and its natural enemies were conducted in the laboratory and field.

Not only were the objectives of this project met, but the information gained was disseminated to growers, students and others by means of several workshops and through presentations at scientific meetings.

Since the conception of the project in 1997 to the end of the funding period, there has developed a sense of National intellectual property rights. The Peruvian government is interested in protecting their native species; however, this hindered researcher's ability to sample grower's fields – collecting permits were required to sample from each Department in Peru. Furthermore, dead material (insect parasitoids) could not be sent to world experts for verification of identification. As such, this necessitated the development of taxonomic expertise, an aspect not anticipated in the original grant proposal. While protection of native species is laudable, independent verification of their identity is critical to further scientific advancement and intellectual exchange on an international level.

I. Research Objectives

The pea leafminer is a major economic pest of potato, beans and other staple vegetables in many parts of South and Central America, where growers spray 5-13 times per season for its control. This pest has recently spread to such distant places such as Israel, Europe, Africa, Malaysia, and Indonesia (Weintraub and Horowitz, 1995) where it is inflicting serious damage. Intensive use of "hard" or non-selective chemicals (carbamate, organophosphate and pyrethroid insecticides) attempt to control the adults. This not only results in higher costs of potato production but has also generated secondary pest resurgence of mites and other pests, necessitating even more chemical applications (Chavez and Raman, 1987). This vicious cycle has generated expenditures that many growers cannot afford, particularly in the third world. Furthermore, it produces toxic residues which impair farmers' health and damage the environment. The introduction of this native South American pest to Israel, Europe, Africa and Asia has created a global problem.

Although *Liriomyza huidobrensis* was identified in 1926 in South America (Blanchard, 1926), a survey of the native predators and parasitoids in Peru was never conducted. That the leafminer could be controlled by releasing parasitoids was demonstrated in European greenhouses (van der Linden, 1991); however, the guild of the native Peruvian species was known, such an undertaking had not been conducted. In Israel, substantial reductions in chemical applications (down from 5 to 0-1 applications per season) have been achieved in potato fields, primarily by shifting away from non-selective adulticides and implementing translaminar larvicides at specific times in the developmental lifecycle (Weintraub, 2001). The potential economic benefits from developing and using native South American parasitoids, with concomitant reductions in hard chemical usage, are of inestimable value to Peruvian farmers.

The research objectives were:

1. Determine native beneficial insect guild for *L. huidobrensis* in Peru, and the extent to which control is exerted by each species.
2. Evaluate the efficacy of translaminar larvicides (new and existing) on pest and parasitoid populations.

3. Develop and implement an integrated control strategy, as such: establish mass rearing procedures for the leafminer and then develop parasitoid rearing methods for mass release.

Liriomyza huidobrensis is one of many pests that have competed for researchers' attention and available funding in South America. This project allowed Peruvian integrated pest management (IPM) scientists to increase their capacity to deal successfully with the leafminer pest by learning to identify its natural enemies, as well as the roles played by the various species of parasitoids in the management of the fly in potato and other local crops. Researchers from a number of disciplines have been conducting trials on various aspects of potato and sweet potato pests, diseases and agricultural techniques for years at the International Potato Center (CIP). This project dovetailed well with on going research activities on resistant potato varieties and provided a strong basis for future research.

The novel approach developed with this grant was to reverse this process of constantly generating new insecticide-resistant pest species by using the leafminer's native beneficial species to reestablish control. Control of the leafminer can be achieved through enhancement of one or more of these naturally occurring beneficial species, and by simultaneously replacing general insecticides with ones that are more selective to the pest. Once this is demonstrated to be successful, dissemination can be world wide since CIP has satellite research institutes in Asia and Africa.

In addition to the US-AID/CDR funds received, this research was additionally supported by the International Potato Center and by the Agricultural Research Organization. Both institutes provided additional funding in terms of staff salaries and facility maintenance. In addition to the formal agreement under this grant, additional research institutions were involved in the following capacities:

- Agreement between CIP and San Agustin National University (Arequipa) to develop the thesis "Seasonal occurrence of the Leafminer fly *Liriomyza huidobrensis* in potato and faba bean crops in the Tambo valley, Arequipa (Peru)" From July to December 2004.
- Agreement between CIP and Tumbes National University (Tumbes) to develop the thesis "Seasonal occurrence of the Leafminer fly *Liriomyza huidobrensis* in bean crops in the Tumbes valley, Tumbes (Peru)" From July to December 2004
- Agreement between CIP, Pedro Ruiz Gallo National University (Lambayeque) and Vista Florida Experimental Station (Lambayeque) to develop the thesis

"Seasonal occurrence of the Leafminer fly Liriomyza huidobrensis in bean crops in the Picsi valley, Lambayeque (Peru)". From July to December 2004

- Agreement between CIP and Valle Grande Rural Institute to develop the research "Evaluation of selective chemical and natural product insecticides to control different stages of LMF and associated parasitoids in field conditions"

II. Methods and Results

Survey of Liriomyza huidobrensis *and parasitoids*

The potato growing season is from May to October. Surveys were conducted at two times of the year: April-May and July-August in all 10 coastal departments (Table I, Figure 1). Potatoes, beans and tomatoes were usually grown in fields of 1-2 hectares. All other vegetables were usually grown in smaller plots – 0.1-0.25 hectares. All fields were commercial and insecticides were applied by the discretion of the individual grower.

If there were leafminers present, 20-30 leaves were randomly selected, from the lower part of the plant, while walking through the field. If no leafminers were present a minimum of 10 leaves were collected. Leaves from each field were collected in paper bags and stored in a cooler. In the evening, these leaves were transferred to plastic containers, 15 x 25 x 10 cm high, in which the top was cut away and replaced by a 20 x 12 cm insect proof screen. The bottom was lined with damp paper toweling to maintain humidity, leaves were separated by dry paper toweling. Toweling was changed every 2 days.

If no parasitoids emerged before leafminer pupation, pupae were collected and stored in plastic Petri dishes until the adult flies or parasitoids emerged. Leaves were returned to the container after pupae were collected. The species and number of all insects were recorded.

Table I. Locations in Peru surveyed for the leafminer, *Liriomyza huidobrensis* and its associated parasitoids.

Department	Province	District	No. of Sites
TUMBES	Tumbes	Tumbes	9
		Zarumilla	1
PIURA	Paita	San Luis de Vichallay	7
	Sullana	Sullana	5
	Sechura	La Unión	1
	Piura	La Arena	2
		Castilla	3
LAMBAYEQUE	Chiclayo	Picsi	11
		Monsefú	2
		Reque	2
		Saña	3
LA LIBERTAD	Ascope	Paiján	4
	Trujillo	Simbal	6
		Moche	7
	Virú	Virú	16
ANCASH	Casma	Casma	5
	Santa	Santa	9
	Huarmey	Huarmey	2
LIMA	Barranca	Barranca	5
		Supe	3
	Huaura	Huaura	1
	Huaral	Huaral	9
		Chancay	3
TACNA	Tacna	Tacna	20
		Sama	2
		Locumba	1
MOQUEGUA	Mariscal	Moquegua	19
AREQUIPA	Islay	Punta Bombón	23
	Arequipa	Cocachacra	6
	Caylloma	Vitor	2
	Camaná	Majes	5
		Camaná	11
ICA	Nazca	Nazca	9

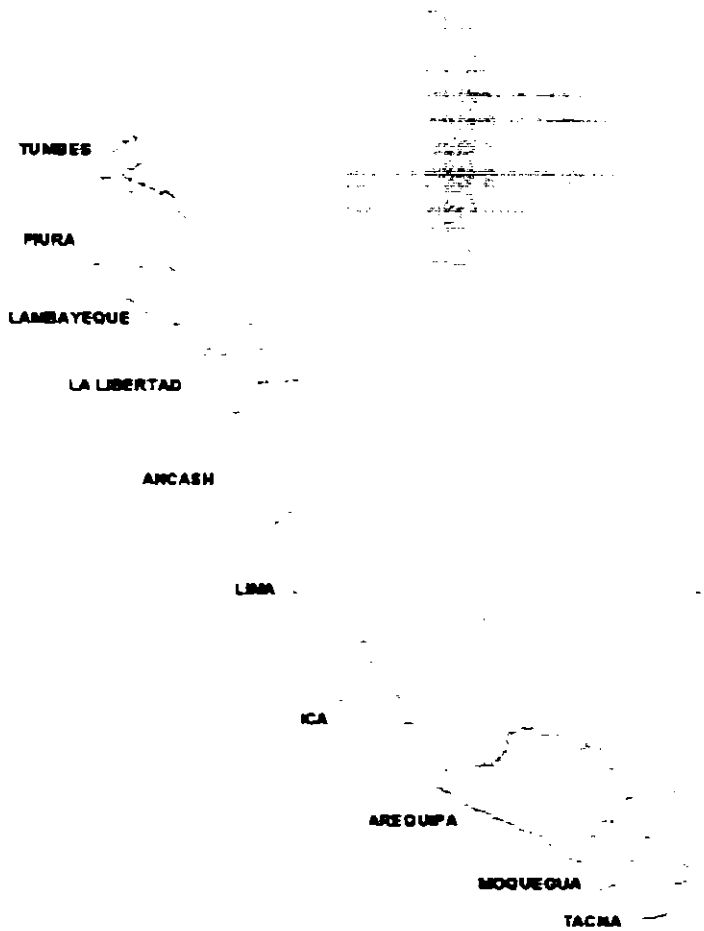

Figure 1. Departments along the coast of Peru (light colored areas) which were surveyed for *Liriomyza huidobrensis* and its parasitoids.

Parasitoids from five families. Eulophidae (11 species), Braconidae (3 species), Eucolidae (1 species), Pteromalidae (1 species) and (Elachertinae (1 species) were found were throughout the coastal regions of Peru (Figure 2). Eulophid parasitoids were predominant in the south, especially in the department of Tacna. Pteromalid parasitoids were predominant in the north, especially in the department of Lambayeque. The department of Ica had the most diversity of parasitoids, with a total of 15 species.

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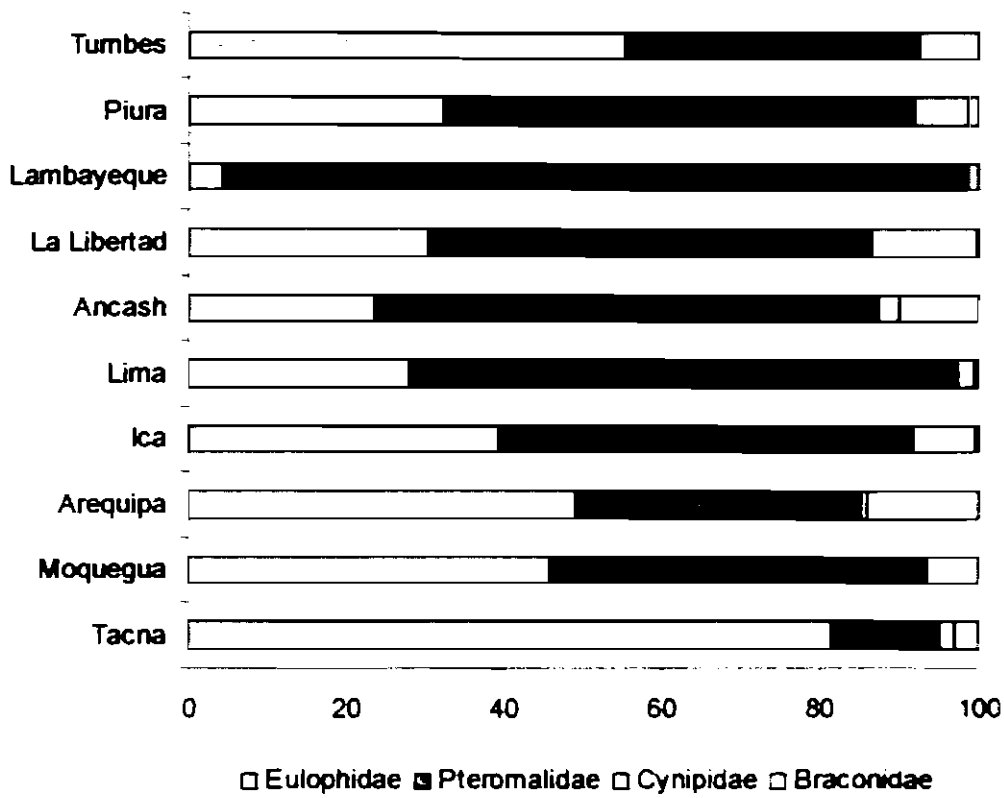

Figure 2. Families of parasitoids found on *Liriomyza huidobrensis* in different costal departments in Peru.

Of the 17 species of parasitoids found, there were 7 koinobiont (a parasitoid developing in a host that continues to be mobile and able to defend itself; hosts that are larvae often are not killed until they have prepared cryptic pupation retreats) species (*C. cariea*, *C. flacilla*, *H. arduine*, *Ganaspidium* sp. and 3 yet to be identified braconid species) and 10 idiobiont (a parasitoid whose host is rendered immobile by the parent of the idiobiont, and the said host is consumed in the location and stage it is in when attacked, or at least in a nearby location to which the parent of the idiobiont has moved it) (*Chrysocharis* sp., *D. websteri*, *D. begini*, 2 *Chrysonotomyia* spp., 4 eulophid spp. and *Zagrammosoma* sp.). Figure 3. Of note, was the fact that in the far north, Tumbes, there was almost 50% of each type; whereas generally there were more koinobiont species found throughout coastal Peru.

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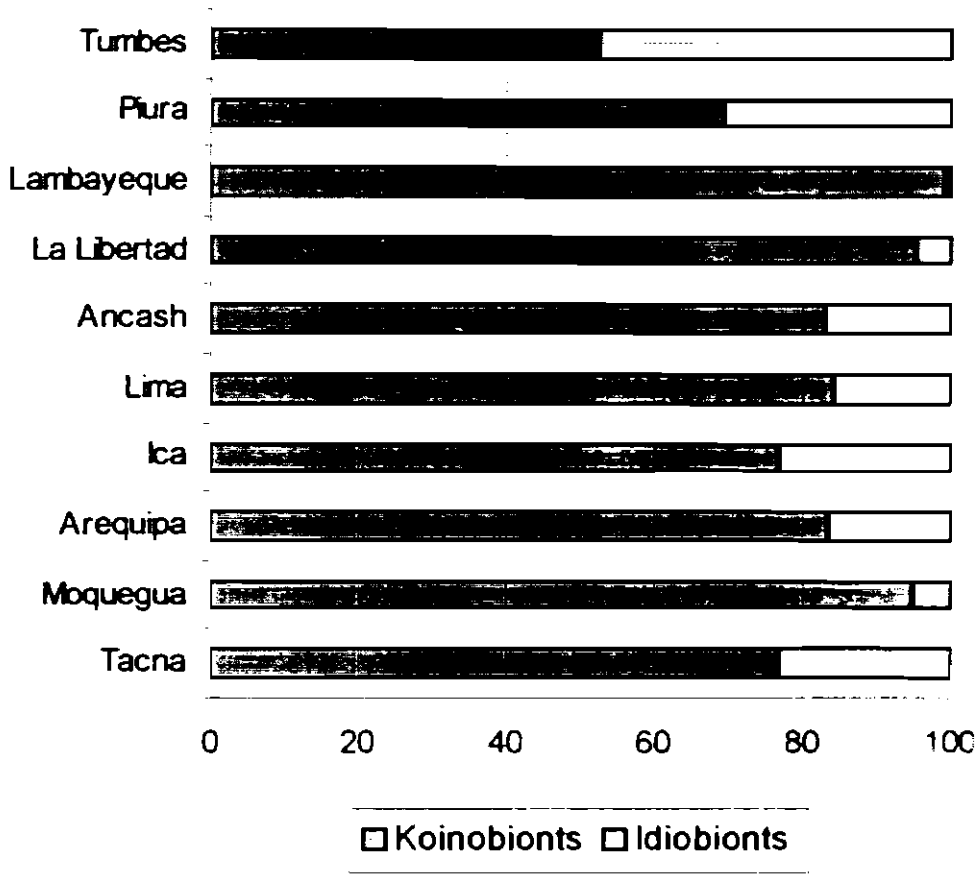

Figure 3. Koinobiont and idiobiont species found on parasitoids of *Liriomyza huidobrensis* in different costal departments in Peru.

There were clear differences in the number and species of parasitoids found on different leafminer host plants. The predominate parasitoids throughout Peru were *Chrysocharis caribea*, *C. flacilla*, *Halticoptera arduine*, and *Digiyphus websteri* (Table 2, Figure 4). *Halticoptera arduine* was recovered from all of the cultivated plants and was the species with the widest distribution in Peru.

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Table 2 Species of parasitoids that emerged from field-collected, *Liriomyza huidobrensis*-infected, crop plant

Plant	*Chrysocharis canibea*	*Chrysocharis flavilla*	*Chrysocharis hedybeo*	*Halticoptera arduenae*	*Ganaspidium* sp	*Phaedrotoma* sp	Braconidae sp B	Braconidae sp C	*Diglyphus wetsteri*	*Diglyphus begini*	*Chrysonotomyia* sp A	*Chrysonotomyia* sp B	Eulophidae sp A	Eulophidae sp B	Eulophidae sp C	Eulophidae sp D	*Zagrammosoma* sp
Beet	+	+		+					+								
Basil	+			+													
Alfalfa	+	+		+	+	+	+	+	+	+	+		+	+	+		
Celery	+			+	+				+	+	+				+		
Peas	+	+	+	+	+				+	+	+		+	+			
Beetroot	+	+	+	+	+				+	+	+						
Chinese onion	+			+					+								
Cabbage	+	+		+	+				+	+		+					
Spinach	+	+		+	+	+			+								
Bean	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Ful	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+
Lettuce	+	+	+	+	+	+											
Corn	+	+	+	+	+	+				+							
Marigold	+			+	+	+				+					+		
Turnip		+		+							+						
Potato	+	+	+	+	+	+	+	+	+	+							
Cucumber	+		+	+					+								
Radish	+	+		+	+	+			+								
Soya	+		+	+	+				+	+	+	+	+	+	+	+	
Tomato	+	+	+	+	+	+	+	+	+								
Italian squash		+		+	+	+					+						
Squash	+	+		+					+	+							

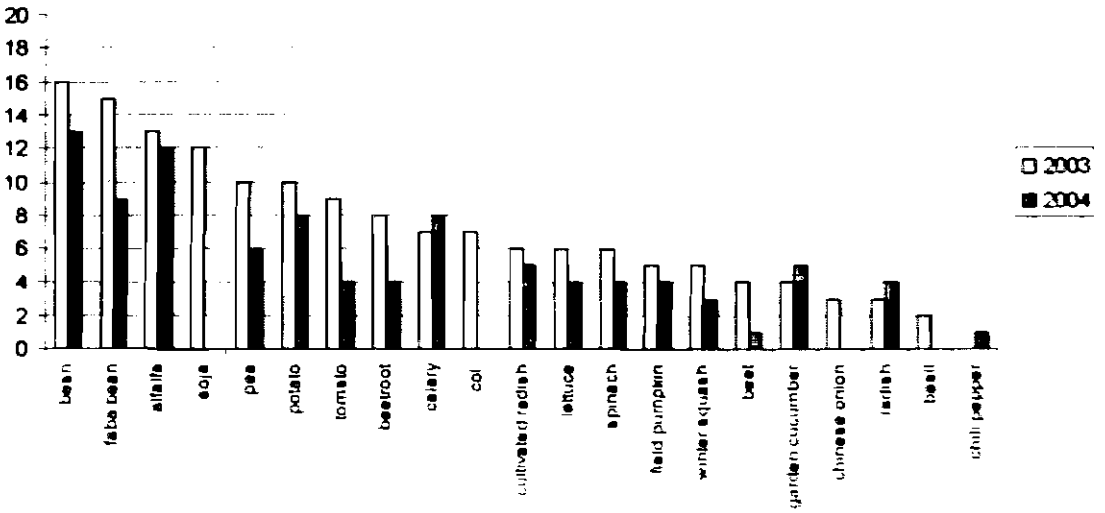

Figure 4. Relative abundance of *Liriomyza huidobrensis* parasitoids on different crop plants.

Seasonal population fluctuations in Peru

This study was carried out in potato fields in La Molina from May to November. Three commercial fields (Amarilis, Revolucion and Tomasa) were evaluated during the autumn, winter and spring seasons. Amarilis was planted in the autumn, and Revolucion and Tomasa in the winter. Each field was divided in three sectors and samples of 20 leaves/sector were collected. Leaves were taken to the laboratory to observe and count emerging leafminer and parasitoids adults sampled. Adult population was monitored with yellow sticky traps (20 x 20 cm). A trap was placed in each sector (3 traps/field). Fields were managed according to normal grower practices for the area.

The leafminer pressure recorded in 2002 represented the normal trend of the populations dynamic in the coast of Peru. Adult population peak occurred in winter (July) and spring (September), with 9075, 15775 and 17038 adults/trap in Amarilis, Revolucion and Tomasa varieties, respectively. These peaks coincided with phenology age of the potato crop. There was a relatively slow increase during the vegetative growth and a rapid and sustained augment during flowering and maturing,

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followed by a decline as plants enter into senescence (Figure 5). Similar population trends were noted for each year (data not shown)

Parasitism: Level of parasitism was very low due to the excessive use of non-selective insecticides (methamidophos). After insecticide applications were stopped in theses fields, the leafminer fly population increased, followed by an augment in the rate of parasitism by indigenous parasitoids. Only five species were recovered in these fields (*H. arduine*, *Chrysocharis* sp., *Diglyphus websteri* and *Ganaspidium* sp.). When little or no insecticides are used, parasitism of LMF larvae during winter (April-August) varies from 35% to 55%. But in early spring (September) total parasitism increases steadily until later spring (November) when percentage of parasitism is near 100%.

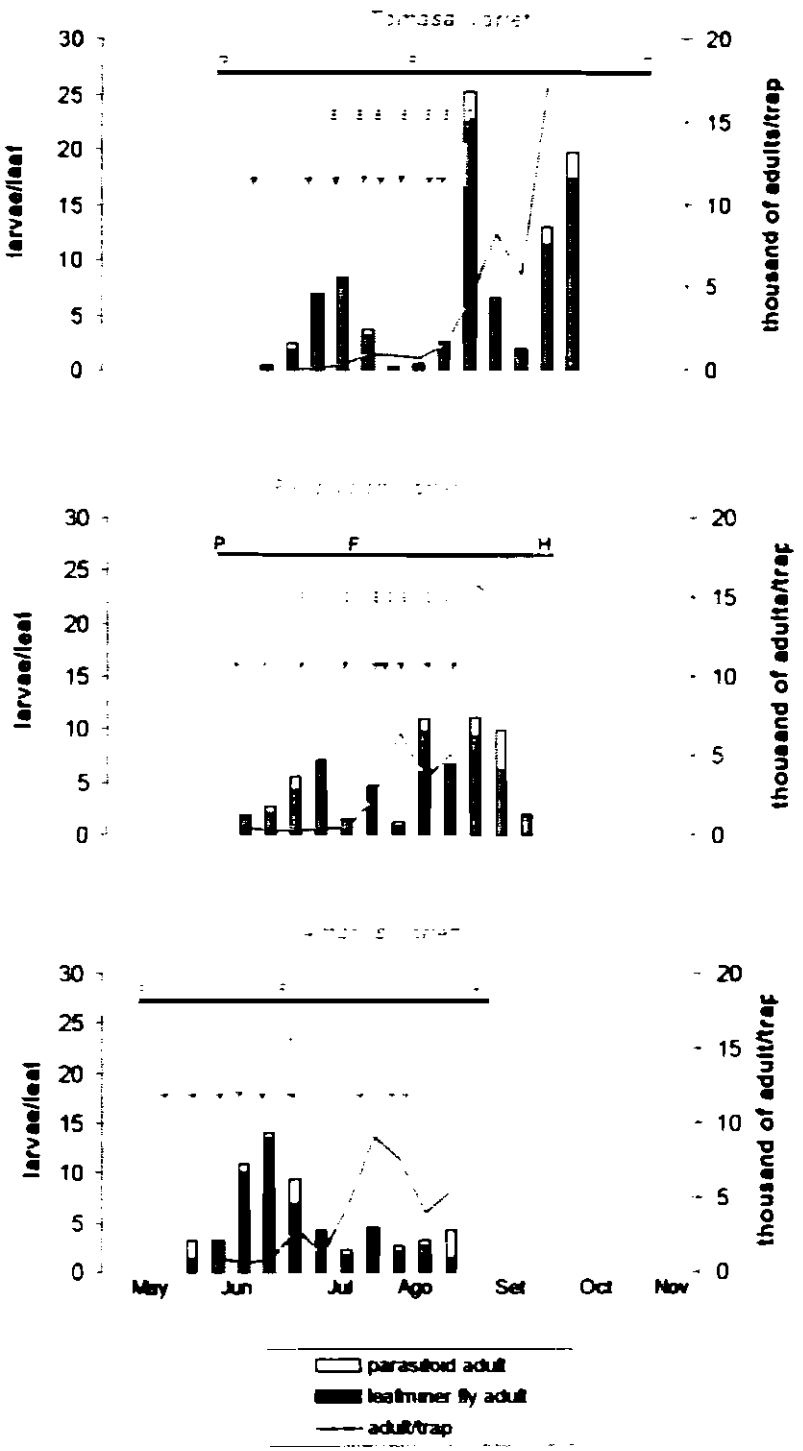

Figure 5. Seasonal populations of *Liriomyza huidobrensis* in three commercial varieties of potatoes. P – planting, F – flowering, H - harvesting

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Survey of L. huidobrensis and parasitoids in Israel

In Israel, parasitoid species were surveyed in two major potato growing areas, the western Negev and the Tel Aviv-Gush Dan region. Plants were sampled weekly by vacuum sampling and by collecting 10 plants from each field throughout the growing season (September – June). In the laboratory, plants were divided into three parts starting from the top 6 leaves, the middle 6 leaves and the oldest remaining leaves. These sections were placed in paper bags and kept at room temperature (ca 25° C) for a minimum of 4 weeks to allow adults to emerge.

Results of the field survey of *L. huidobrensis* infested potato plants is by vacuum collection is shown in Table 3. A total of 12 species were recorded. A greater variety of species were found in the western Negev; potato is one of the primary autumn-spring crops and fields are usually several hectares in size.

Table 3. Percent of parasitoid species collected by vacuum sampling potato fields between 2002-2004.

Species	Center	Western Negev
Diglyphus isaea	48.4	64.4
D. crassinervis	1.6	0.6
Neochrysocharis formosa		1.3
Opius sp		25
Platynocheilus cupritrons	2.6	1.9
Pnigalio soemius		1.3
Chysocharis pentheus	7.9	3.1
Hemiptarsenus zilahisebessi	4.8	0.6
Neotrichoporoides sp.		1.3
Pteromalus sp.	12.7	0.6
Trichomalopsis sp.	12.7	0.6
Scelionidae	7.9	

When the leafminer was first found in Israel the only parasitoid found was *Diglyphus isaea* (Weintraub and Horowitz, 1995). At the beginning of this survey, 10 years after L. huidobrensis arrived in Israel, *D. isaea* was the predominant, but not exclusive parasitoid. While all of the parasitoids in Table 3 attack the leafminer, only 7 species (Table 4) were found emerging from pupae. An *Opius* sp was a predominant parasitoid in 2004 in the center and western Negev regions.

Table 4 Species and percent of parasitoids recovered from *Liriomyza huidobrensis* pupae collected from potatoes, divided into 3 plant sections, in two regions of Israel in 2002 - 2004

		2002		2003		2004	
Species	**Plant height**	**Center**	**Western Negev**	**Center**	**Western Negev**	**Center**	**Western Negev**
Diglyphus isaea	Top	8.2	3	14.3	0.7	0.7	0.43
	Middle	9.7	47	14.3	2.9	0.7	1.3
	Bottom	28	37.3	61.9	4.4	0	0.43
Neochrysocharis formosa	Top	6.5	2.2				
	Middle	17.2	1.5				
	Bottom	15	1.5				
Opius sp	Top	1.1	3.7	0	21.7	21.5	43
	Middle	7.5	2.2	4.8	31.9	74.5	51.1
	Bottom	10.8	1.5	4.8	30.4	2.5	0
D. crassinervis	Top				0		
	Middle				2.2		
	Bottom				3.6		
Trichomalopsis sp.	Top				0		
	Middle				0		
	Bottom				0.7		
Hemiptarsenus zilahisebessi	Top				0		0
	Middle				0.7		0.43
	Bottom				0.7		0
Sympiesis sp.	Top						0
	Middle						2.6
	Bottom						0.43

Mass rearing of Liriomyza huidobrensis and parasitoids in Peru

Once it was established that the leafminer could be easily reared on bean plants, a mass rearing technique was developed. Various life parameters of the leafminer were determined to optimize the conditions for the mass rearing. The effects of temperature for first examined. Females were held at 5 different temperatures (15, 18, 20, 22, and 26° C) and the number of eggs laid, feeding punctures and longevity were determined. Bean seedlings were used as the host plant. Polyethylene vases (1000 cc) were used as LMF rearing chambers. On the top, they had a hole (6 cm diameter) to permit the pass of air. Inside each vase a tube of glass (50cc) was introduced. It was filled with water to keep the plant alive during the experiment. A couple of recently emergency flies were released within each vase. The leaves were changed every 24 hours until LMF females were dead. The number of feeding punctures, eggs and the survivor of females was evaluated.

Trials were replicated with at least 20 females at each temperature. Flies were held at different densities (2, 4, 6 or 8 pairs), and the number and weight of pupae were determined.

The maximum number of eggs were laid at 18° C; a total of 169 eggs at a rate of 5.5 per day (Table 5). However, at this temperature, peak oviposition didn't occur until 16 days after oviposition began. At 22 and 26° C, peak oviposition occurred at day 4. This coincided with the peak number of feeding punctures on the leaves, 315 and 459 for 22 and 26° C, respectively.

In the laboratory, under controlled temperature (20°C), four different densities of LMF infestation were evaluated. As an infestation unit, a plot with five plants of bean (*Vicia faba* 30 days old) was considered. In a rearing cage, six plots were arranged. Couples of recently emerged LMF male and female were separated. Densities of 2, 4, 6 and 8 pairs by plot were released inside each rearing cage, with three replicates per density. Every three days (during 14 days), plots were removed from the cages and taken to the greenhouse, to complete the LMF cycle. After 15 days, pupae were recovered.

Table 5. Oviposition activity of *Liriomyza huidobrensis* at 5 temperatures

Temp. (°C)	n	Means number of eggs per female		Peaks oviposition		Means number of feeding punctures per female		Peaks feeding		Longevity
		Total	Per day	# eggs	day	Total	Per day	# punctures	day	(days)
15	26	72,7	1,0	8,3	21st	2040,7	28,7	119,2	7th	35,9
18	20	169,2	5,5	14,4	16th	3081,4	78,0	234,0	5th	24,4
20	26	112,2	2,4	11,8	11th	2199,6	47,8	206,1	7th	22,5
22	20	111,2	3,6	17,2	4th	2894,8	93,4	315,0	4th	21,4
26	20	88,6	3,4	19,0	4th	2593,0	92,6	459,0	4th	16,8

Number and weight of pupae are showed in Table 6. The highest pupae production was obtained in the treatment with 6 and 8 couple/host unit (5847 and 5429 pupae respectively), without finding differences between them. A clear positive relationship between the infestation level and number of recovered pupae was observed. On the opposite, the relationship between the number of recovered pupae and the weight/pupae was negative.

Table 6. Egg production and adult emergence from different pair densities of *Liriomyza huidobrensis* on bean plants.

N° couples	Infestation times						Total production		Adults emergency (%)
	1st to 3rd day	4th to 6th day	7th to 10th day		11th to 14th day				
2	622	1383	923	b	565	b	3493	c	90.3
4	621	1571	1244	ab	1019	ab	4456	bc	89.9
6	741	1807	1652	a	1229	a	5429	ab	88.9
8	773	1941	1675	a	1458	a	5847	a	90.7

Based on the results above mass rearing is now being conducted as follows Faba bean (*Vicia faba*) plants are grown in screenhouses under no controlled conditions. In plastic pot, four seeds are planted in a 1:1:1 mixture of soil, sand and moss. Plants are ready to use as hosts when the stems are approximately 20 cm tall. The time required to achieve this size varied in the different season, being between 20 days in summer (25°C) and 30 days in winter (15°C). 300 pots were prepared each 15 days. Infestation of leafminers takes place in a rearing room at 20°C. Nine rearing cages (55 cm wide x 100 cm long x 75 cm high) are used for production, ten pots with faba bean plants are placed in each cage, 300 newly emerged LMF adults (mixed sex) are released. Every three days (three times) pots are replaced with new plants. The infested plants are placed in another rearing room at 25°C for 4 days to complete larval stage (part of this larval production is used to rear parasitoids colonies). After that, plants are cut on the bottom and laid into plastic containers covered sand to facilitate the harvest of pupae.

Mass rearing of Halticoptera arduine

Four commercially faba bean (*Vicia faba*) seeds were planted in 10 x 10 cm pots and held on greenhouse benches for about 20 days. In a rearing room (20°C), six pots are exposed to adult leafminers (3 days old) in colony cages (55 wide x 100 long x 75 high cm) for oviposition. After 24 hours, plots are removed from the cages. Plants containing late second to early third instars are exposed to adult *H. arduine* (300 adults) in parasite colony cages for 48 h. After 20 days to 25°C, an average of 3500 adult parasitoids are recovered per cage.

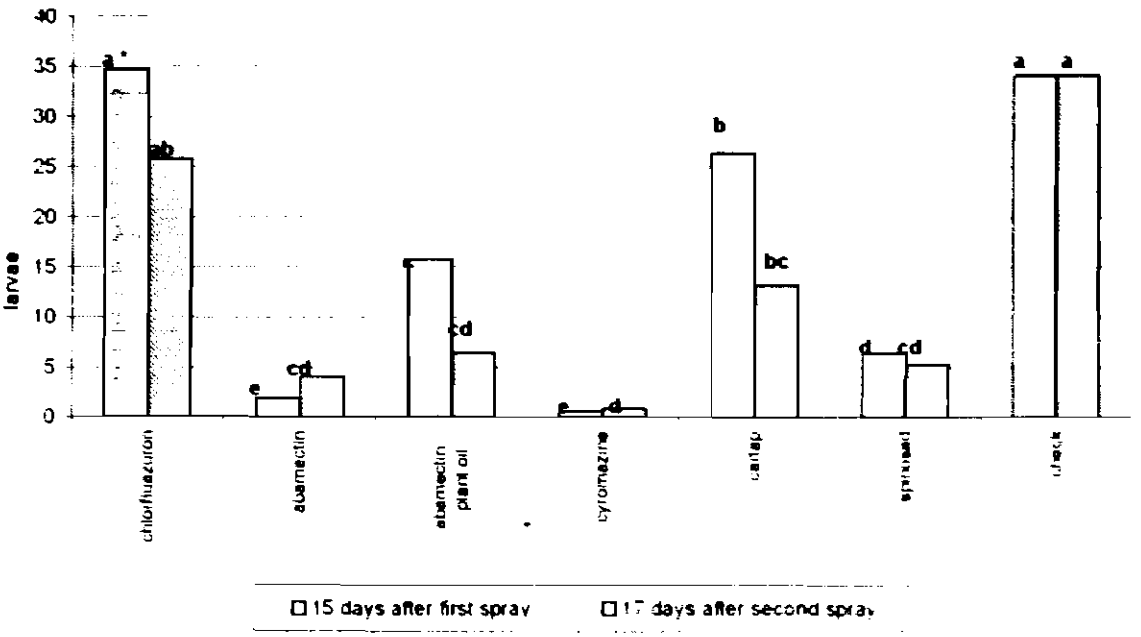
Additionally, colonies of the following parasitoids are being maintained
Endoparasitoids: *Chrysocharis flacilla*, *C. caribea*, *Chrysocharis* sp, *Opius* sp
Ectoparasitoids: *Diglyphus websteri*, *D. begini*.

Effect of translaminar Insecticides on Liriomyza huidobrensis *larvae*

Trials were run in field potatoes (in Peru) and in potted plants (in Peru and Israel). The field trials (Peru) included the following insecticides: cyromazine, abamectin, clorfluazuron, and cartap. The experiments on the effect of cyromazine, abamectin, chlorfluazuron, cartap, and an absolute control (no insecticides) on leafminers were conducted at CIP's HQ station in a winter season (July-October 2002). Cyromazine and abamectin were the most effective insecticides against leafminer larvae with 0.6 and 1.9 larvae/leaf respectively, 15 days after first spray. The treatments, spinosad and abamectin mixed white plant oil registered an average of 6.5 and 15.8 larvae/leaf respectively. No significant differences in number of LMF larvae were observed between chlorfluazuron (34.8 larvae/leaf) and the check (34.2 larvae/leaf). In the second spray, cyromazine registered 0.9 larvae/leaf 15 days after spray. No significant differences were found between abamectin (4.1 larvae/leaf), abamectin + vegetable oil (6.5 larvae/leaf) and spinosad (5.3 larvae/larvae) (Figure 6). This study leads us to conclude that cyromazine, abamectin and spinosad can be alternated for better leafminer control in the field.

Figure 6:

Figure 1. Effect of insecticides on LMF infestation under field conditions. La Molina-Peru, 2002.



* The same columns followed by a common letter are not significantly different

The experiment was conducted at CIP's HQ station in Lima (Peru) in a winter potato crop (July-November). Treatments included: cyromazine (Trigard 75PS), abamectin (Vertimec 1.8CE), chlorfluazuron (Atabron 5CE), cartap (Padan 50PS), spinosad (Tracer 120SC), imidacloprid (Confidor 350SC) and an absolute control (no insecticide).

Experimental design.- A randomized complete block design with four replicates. Experimental plots had 6 rows wide with 20 m long each (105 m2). Plots were treated 60 days after planting with a motorized backpack sprayer at a rate of 300 liter/ha. The four central rows of each plot were considered for evaluations.

Sampling methods. – Samples were collected from the four central rows. Five leaves by plot were randomly collected and taken to the laboratory to emergence of LMF adults or parasitoids. Collections were done 0, 4, 8, 12, 16 and 20 day after spray. *Statistical analysis.*- Before statistical analysis, data were transformed (square root + 1) and analyzed using 1-way analysis of variance (ANOVA). Means were separated by Waller-Duncan test (P=0.05). In order to correct mortality differences of larval infestation before application, Abbott's formula was used

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Cyromazine was the most effective insecticides with a high larval mortality until 20 days after spray, followed by abamectin and spinosad (Figure 7). The parasitoids complex constisted of 8 species: Pteromalidae (*Halticoptera arduine*). Eulophidae (*Chrysocharis caribea*, *C. flacilla*, *Diglyphus websteri*, *D. begini* and *Chrysonotomyia* sp.), Cynipidae (*Ganaspidium* sp.) y Braconidae (*Opius* sp.). Before and after applications *H. arduine* seemed to be the most important parasitoid on the field. The parasitoid population was reduced by chlorfluazuron, cartap, spinosad, and imidacloprid until 8 days after application. Twelve days were needed to recover the normal level of parasitoids in these treatments. Ectoparasitoids (*Diglyphus* spp. and *Chrysonotomyia* sp.) were the most susceptible to chlorfluazuron and imidacloprid applications. Both, the amount individuals by specie and the number of species were affected by cyromazine. In the other hand, the opposite results were showed by abamectin. This study leads us to conclude that abamectin and spinosad can be alternated with cyromazine for better LMF control in the field and maintaining the parasitoids population.

The effect of the botanical insecticide *Sapindus saponaria* on leafminer adults was evaluated under lab conditions in Peru. Crude ethanol extracts of *Sapindus saponaria* (Sapindaceae, soapberry tree) fruits (Figure 8) were obtained. This botanical has previously been shown to have insecticidal activity (Alonso-Amelot et al., 1994). Three concentrations of each extracts were applied on potato plants. In the greenhouse, plants were put into rearing cages and LMF adults released. Effect on adult feeding was evaluated. Potato plants sprayed with ethanol extracts of *S. saponaria* fruits showed 47.8% less leafminer feeding punctures, against 23.4% observed in crude extracts. More tests are necessary to consider this natural product as an alternative to synthetic chemical formulation against LMF adults.

Figure 7

Figure 2. Effect of six insecticides on abundance of LMF hymenoptera parasitoids. La Molina, 2003.

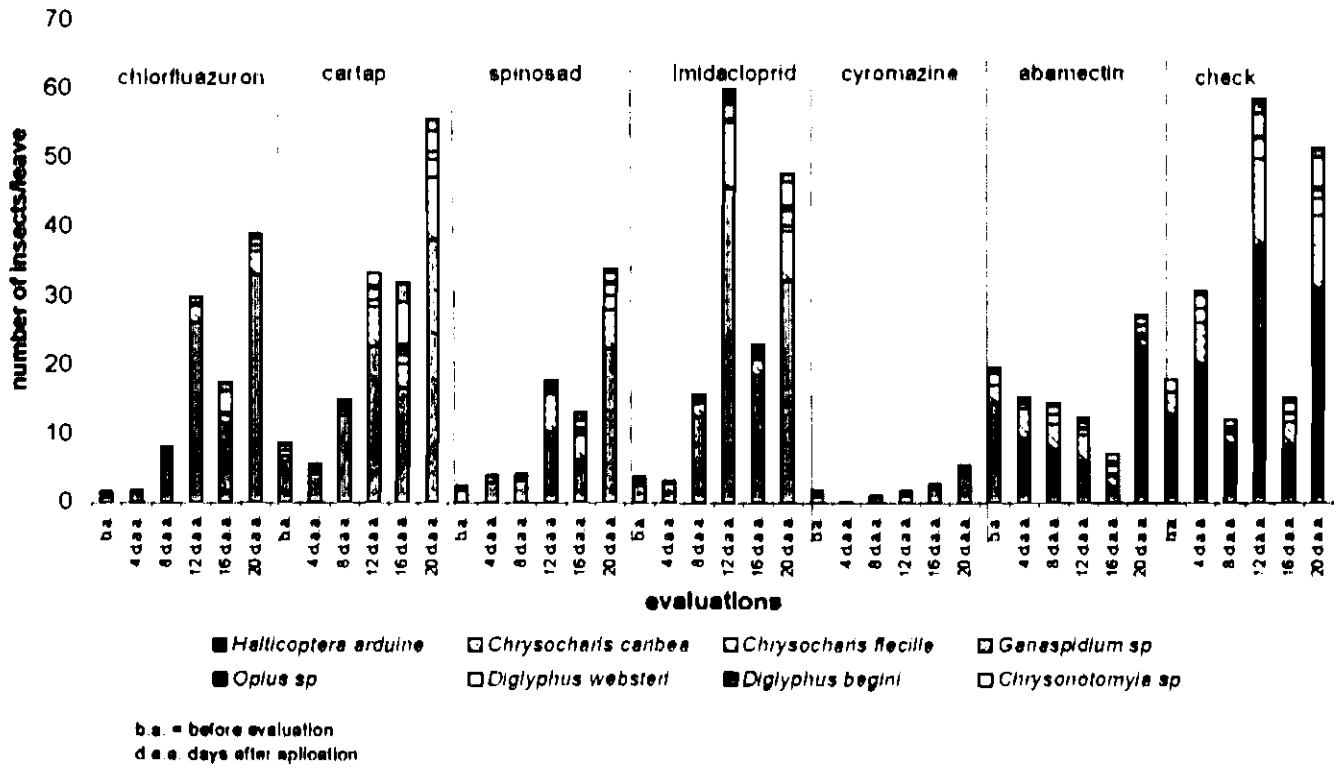

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Figure 8 *Sapindus saponaria* fruits and leaves.

Effect of two strains of *Paecilomyces fumosoroseus* on leafminer adults were evaluated under lab conditions in Peru. Fungus concentrations (108, 107, 106, 105, 104 and 103 conidia/ml) in water suspension were evaluated for two isolates of *P fumosoroseus* and one of *Beauveria bassiana*, by exposing adults to conidial suspension. A completely randomized design was used with seven treatments (6 concentrations plus a control) and ten replicates of 10 specimens. Medium infective concentration (LC50) was estimated. The infectivity was estimated as the medium infective concentration (IC_{50}), being 4.79 x 10^7 conidia/ml for *Paecilomyces fumosoroseus* I, 1.01 x 10^9 conidia/ml for *Paecilomyces fumosoroseus* II and 3.80 x 10^7 conidia/ml for *Beauveria bassiana* 210 The most promising strain was *P. fumosoroseus* I, which is the only one that showed some activity even in low concentrations.

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In Israel, potted bean plants were treated with three concentrations of spinosad (Tracer, imported to Israel from DowChemical by Agrochem, Petach Tikva) either by soil drench or by leaf dip. Bulgarian variety beans (*Phaeolus vulgaris*) were seeded in 10-cm-diam. pots (4-6 per pot) with holes in the bottom, in soil consisting of equal parts peat moss, vermiculite and sand. Plants were grown at 23 ± 3° C, with a photoperiod of 14:10 (L:D), until two true leaves were fully expanded, unless otherwise stated. In all trials, plants were placed for ca. 3 h in a cage with a small number of adults from the stock *L. huidobrensis* colony. The short oviposition access period allowed for synchronous egg hatch and subsequent larval development. After oviposition, plants were removed and maintained in a growth chamber at 23° C and a photoperiod of 14:10 (L:D). In trials involving egg treatment, eggs were counted immediately after pots were removed from the stock colony of adults. In trials involving treatment of the three stages of larvae, the number was counted immediately before treatment by using a stereo-microscope with sub-stage lighting.

Spinosad (Tracer) was used in all bioassays and was applied to the soil or leaves of bean plants. In the leaf-dip trials, soil was first watered to runoff, and then covered with thick layers of paper toweling to prevent spinosad dilutions from dripping off the leaves onto the soil, and thereby causing a systemic effect. The leaf dip consisted of water control, 24 ppm, 48 ppm or 96 ppm active ingredient (a.i.) spinosad. Leaves were dipped for 10 sec. For the drench, plants were watered 2 days before spinosad application. Soil was drenched with either 200 ml water (control), or 200 ml containing 12 mg a.i., 24 mg a.i. or 48 mg a.i. spinosad. Plants with eggs, or first, second or third instar larval leafminers were treated. To avoid possible adverse effects from the larvae being crowded on the leaf and stressed because of inadequate food, only leaves with less than 10 eggs or larvae were used. The number of individuals surviving to the subsequent stage was counted until pupae emerged. Paper envelopes were fashioned around leaves at last third instar to catch falling prepupae and prevent them from falling onto the soil or being lost. Pupae were placed in Petri dishes to observe adult emergence. Each trial was repeated until a minimum of 70 individuals were treated per concentration and application regime. Percent survival data were transformed by arc-sin before analysis.

Data were analyzed using two-way analysis of variance (ANOVA), and means were separated using Tukey's HSD quantile function, using JMP5 0 1a (11)

For the leaf dip (Figure 9, LE, L1, L2, L3), all three concentrations of spinosad were most efficacious when applied to the egg stage (df = 3, 99, F = 290 0, P < 0 0001) Death occurred primarily in the first instar; presumably shortly after emergence from the egg since observed tunnels were usually less than 3 mm long. Only with the lowest concentration did some first instar larvae survive to second instar With treatments at successively older larval instars, the spinosad was slightly less efficacious, only the highest concentration was significantly efficacious when third instar larvae were treated (df = 3, 117, F = 3.57, P = 0.016). Results of the soil drench are shown in Figure 10 (DE, D1, D2, D3). In general, larval response (mortality) to the spinosad soil drench was slower; this because the insecticide was being taken up by the plant and acted in a systemic fashion. When the soil of plants with leafminer eggs was drenched, 80-90% of the first instar larvae were killed with the two highest concentrations; however with the lowest concentration only at the third instar stage were > 95% of all treated larvae killed Treatment of the third instar larvae yielded significant results (df = 3, 73, F = 46 65, P < 0.0001); however in the lowest concentration ca 50% of the adults survived

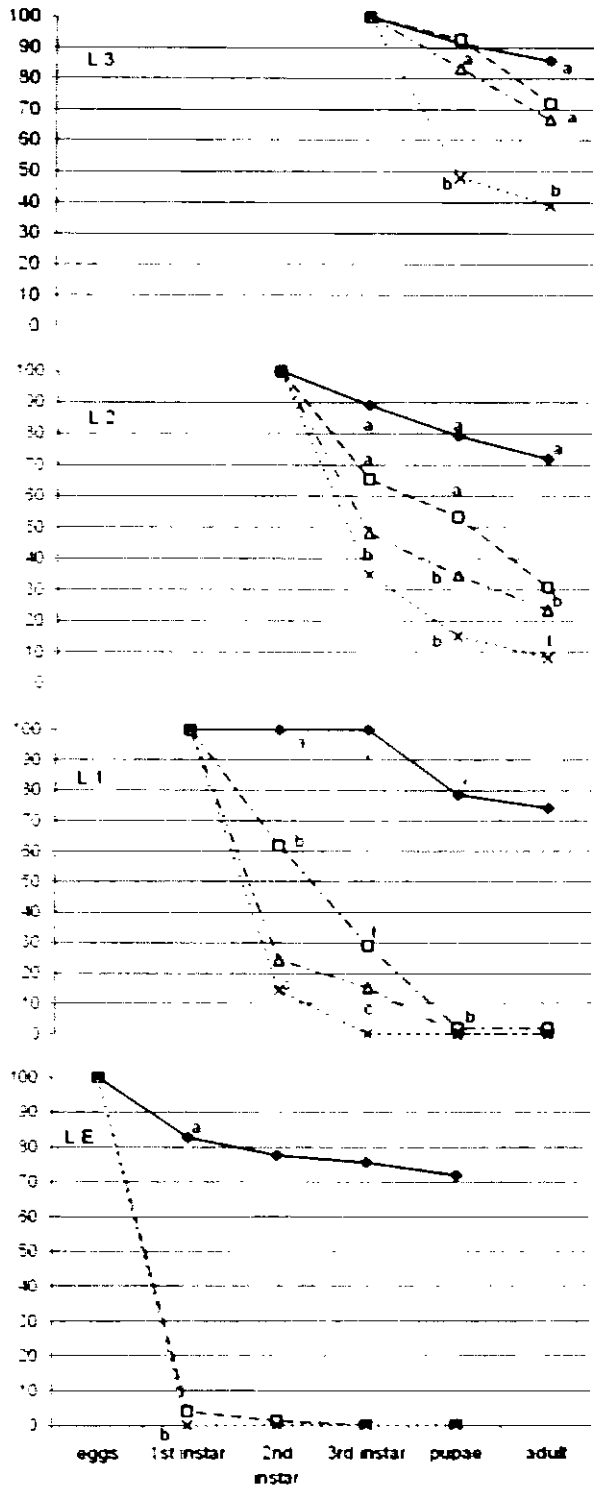

Figure 9. Survival curves after leaf dip of beans containing leafminer eggs (LE), first (L1), second (L 2) or third (L 3) instar larvae. Control (solid diamonds), 24 ppm (open square), 48 ppm (open triangle) and 96 ppm (X).

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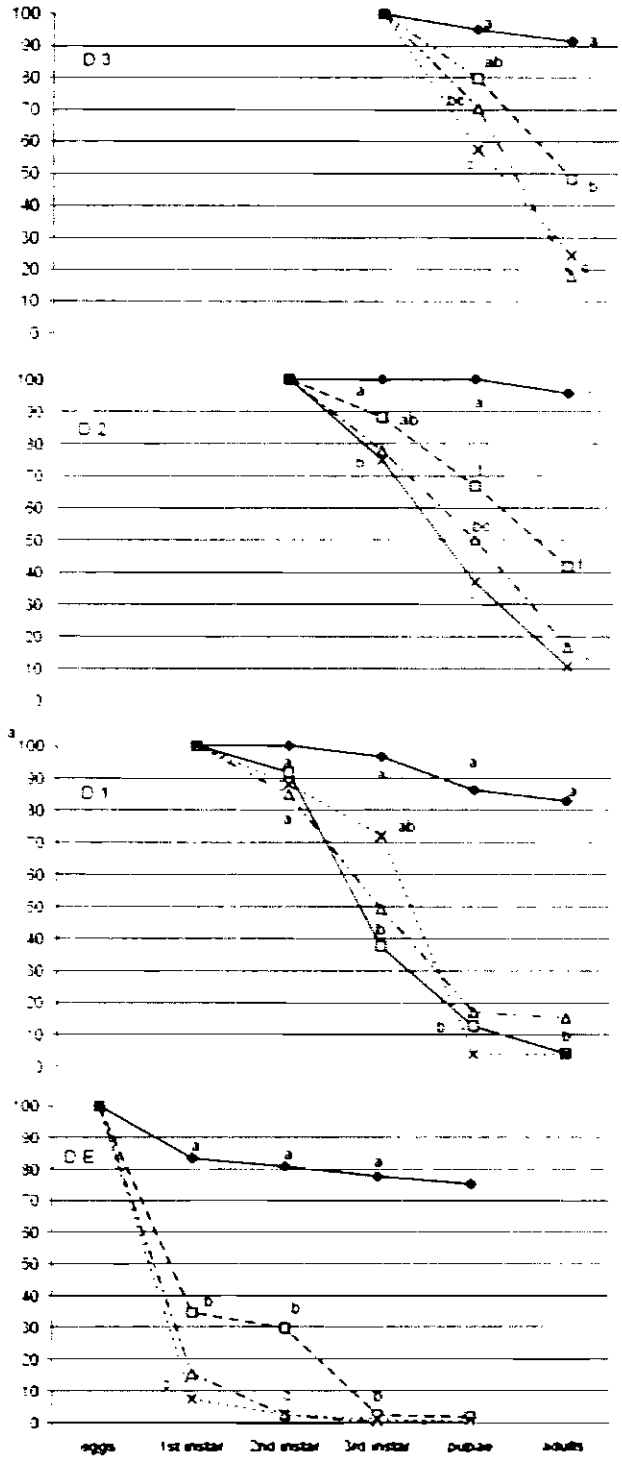

Figure 10. Survival curves after soil drench of bean plants containing leafminer eggs (DE), first (D1), second (D 2) or third (D 3) instar larvae. Control (solid diamonds), 12 mg (open square), 24 mg (open triangle) and 48 mg (X).

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III. Impact, Relevance, and Technology Transfer

Although work on the leafminer has been in progress for many years at CIP in Peru, the general approach has been on developing resistant potato varieties. As a result of this USAID-CDR project, a systematic approach was undertaken to determine the extent of the environmental and botanical (in terms of economically important crops) range of this pest and the associated parasitoid guild. The findings in this study therefore, serve and a concrete basis, a base line, for future research.

Now that the guild of parasitoids is known and the most effective maintained in culture, CIP can move into production and distribution to Peruvian growers. The leafminer is maintained at a level to support commercial production of one parasitoid, *Halticoptera arduine*, which is deemed to be the most generally effective. Several other parasitoids are currently maintained in colonies which can be expanded and distributed.

This parasitoid, *H. arduine*, in combination with the results from the insecticide studies, will allow growers to control the leafminer and significantly reduce the use of insecticides which are particularly destructive to biological control agents. The results of the trials over the past 3 years have been distributed through publications, workshops and personal contact with key growers.

This project has directly impacted directly on a number of individuals, and institutions in Peru. A number of students, who worked on various aspects of this project – primarily in taking national survey data, were supported and have gone on to complete their degrees:

- Silvia Castro, Biologist student. Agrarian National University, Lima (Peru). From June 2004 to April 2005
- Vilma Hualla, Biologist student, San Agustin National University, Arequipa (Peru). From April 2003 to January 2004
- Sandy Prudencio, Biologist student, Federico Villareal National University, Lima (Peru) from July 2003 to April 2004
- Dennis Erikson, Biologist student, Swedish Univ. of Agricultural Sciences, from October 2003 to January 2004
- Margot Huicho, Biologist student, San Marcos National University, Lima (Peru). From June 2004 to September 2004

- Nelida Leyva, Biologist student, Federico Villareal National University, Lima (Peru). From July 2003 to April 2004

In addition to the students listed above, one woman received particular support and is currently writing her thesis on the effect of the primary parasitoid on the leafminer Sandy Prudencio, Biologist student, Federico Villareal National University, Lima (Peru). Thesis: Biologia, comportamiento y establecimiento de un modulo de crianza de *Halticoptera arduine* (Hymenoptera:Pteromalidae) parasitoide de *Liriomyza huidobrensis* (Diptera:Agromyzidae). From 26 April 2004 to 24 July 2005. Her results will show just how efficacious the parasitoid is and will be published in such a fashion that growers will have the results available to them.

As listed in the Research Objectives, a number of institutions have been involved, tangentially, in this research. They were contacted for the national survey. This contact has served to strengthen ties between institutions and generate cooperative research efforts.

Now that the project is complete, CIP has gained extensively in materials and experience. The material benefits are listed below, the experiential benefits are listed in Project Activities/Outputs.

1. Built three rearing rooms with the following dimensions: 2.51 m high, 2.46 m wide and 4.55 m deep. Each one has air conditioner equipment (York), model MOC12P16A; with 12000 Btu/h cold only, 208/230/1/160, split system.
2. Nine insect wood cages of 55 cm wide x 100 cm long x 75 cm high
3. A Microprocessor controlled low temperature illuminated incubator (model 818, Thomas Scientific)
4. Three Olympus stereoscopes (model SZ4045) with light sources and all supplementary
5. An Olympus digital camera (model C4040) with adapters to stereoscope
6. Two steel Cornell cabinets (2500 Series) with 12 Cornell drawers each one, and with Cornell unite pinning trays complete
7. A Cornell open-faced cabinet for 12 drawers, unassembled (2512KD model), with 12 Cornell Tray for vials and complete unit trays and unit vial tray with partitions

8. A countertop open-faced cabinet (2106KD model) for 6 drawers, with all supplementary

IV Project Activities/Outputs

A. Meetings

The first meeting between the participants was held 13 – 18 October, 2002 at the CIP headquarters in Molina, Peru. The Israeli team visited with Dr Lagnaoui and Dr Mujica and toured the facilities there, and observed the on going field trials. Dr. Weintraub gave a lecture on the leafminer fly in Israel. The following two days the Israeli team traveled to and toured the Canente Valley with Dr. Mujica, a major agricultural region in the southern area of the Department of Lima. This is the only valley that has water, because of the Canete River, year round. Two agricultural schools, a research station where beneficial organisms are produced for release in biological control programs and, of course, the field research sites were visited. Several natural enemies, both predators and parasitoids, were observed. Additionally we observed growers implementing the use, developed by Dr. Mujica, of an effective physical control method. Yellow plastic sheets, covered with vegetable oil, were carried through the fields trapping thousands of leafminer flies. Stationary yellow traps (50 x 50 cm) were employed in other fields in the area. The following day we traveled north of Lima to the Chancaylo Valley in Huaral, another large potato growing region. Here potato harvest in addition to visiting several fields were observed. Returning to Lima, there was a follow-up meeting, reviewing the work accomplished and future trials were discussed.

The second meeting between the participants was held 6-8 December 2004 at the CIP headquarters. At this time Dr. Phyllis Weintraub met with the new director Dr. Jurgen Kroschel and Dr. Norma Mujica. We reviewed research progress.

Unfortunately due to the political situation the Peruvian team never traveled to Israel.

B. Training

1. Of CIP personnel

2002

1. Curso de Ecosistema de zonas aridas , 27-31 Mayo 2002. Maestria Ecologia Aplicada de la Escuela de PostGrado UNA

2. Curso de Ecologia Cuantitativa, 12-19 Julio Maestria Ecologia Aplicada de la Escuela de PostGrado UNA

2003

1 Individual training about tritrophic interactions (Plant-pest-parasitoids). Centro de Investigaciones Entomológica de la Facultad de Ciencias Exactas, Fisicas y Naturales Universidad Nacional de Córdoba, Argentina, from 24 to 28 February 2003

2. Course: "Herramientas moleculares para el estudio de la diversidad genetica". 19-23 Noviembre 2003. Maestria Ecologia Aplicada de la Escuela de PostGrado UNA

2004

1. Insect Rearing Workshop, March 21-26, 2004; Mississippi State University, USA

2. Course "Modelización en Sistemas Ecológicos" July 5-9 2004, UNALM, La Molina, Peru

2. Training Activities completed (workshops, individual training, seminars) provided by CIP

1. A workshop for socioeconomic evaluation and identification of LMF species and parasitoids was held in CIP Lima to national organizations from Peru, between August 31 to September 3, 2004; with 13 participants from: Tumbes (Tumbes National University), Lambayeque (Pedro Ruiz Gallo National University), Arequipa (San Agustin National University), Barranca (Pativilca Irrigation Committee) and Cañete (Valle Grande Rural Institute).

2. Individual training about Identification of LMF parasitoids to Laura Niño from FONAIAP (Venezuela) was conducted in CIP Lima.

3. Group training about LMF evaluations in the field and identification of parasitoids was held to government organizations PROINPA in Cochabamba (Bolivia) and INIAP in Carchi (Ecuador).

4. Individual trainings to undergraduate students about LMF and parasitoids evaluations in the field were conducted in Tumbes (Tumbes National University), Lambayeque (Pedro Ruiz Gallo National University) and Arequipa (San Agustin National University)

C. Publications and patents

1. Mujica, N. (2002). Development, integration and promotion of Integrated Management component of LMF. Activity Report, period: January 1, 2002- December 31, 2002. International Potato Center, 16 p.

2. Mujica, N. (2003). Development, integration and promotion of Integrated Management component of LMF. Activity Report, period: January 1, 2003- December 31, 2003. International Potato Center, 11 p.

3. Mujica, N. Espinoza, J. Lagnaoui, A. (2003). Integracion de clones resistentes e insecticides selectivos en el manejo de la mosca minadora *Liriomyza huidobrensis* Abstracts of XLV Congress of the Entomological Society of Peru, 1-5 December 2003. Ayacucho (Peru). p. 161.

*4. Zevallos, R., Mujica, N. and P. Weintraub (2003). Efecto de la temperatura en la oviposicion, alimentacion y longevidad de *Liriomyza huidobrensis* (Diptera: Agromyzidae). Abstracts of XLV Congress of the Entomological Society of Peru, 1-5 December 2003, Ayacucho (Peru). p. 159.

5. Mujica, N., Leiva N., Prudencio C., and O. Ortiz (2003). Efecto de insecticides sistémicos y translaminares sobre *Liriomyza huidobrensis* (Diptera: Agromyzidae) y sus hymenoptera parasitoids. Abstracts of XLV Congress of the Entomological Society of Peru; 1-5 December 2003, Ayacucho (Peru)., p. 138

*6. Mujica, N., Weintraub, P., Hualla, V. and C. Prudencio (2004). Distribucion y diversidad de la mosca minadora *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae) en hospederos cultivados en la costa del Peru. Abstracts of XLVI

Congress of the Entomological Society of Peru; 7-11 November 2004, Arequipa (Peru). p. 61

7. Mujica, N. and A. Lagnaoui (2004). Efecto de la densidad de adultos de *Halticoptera arduine* (Hymenoptera: Pteromalidae) en el parasitismo larval en *Liriomyza huidobrensis* (Diptera: Agromyzidae). III Congreso Latinoamericano de la Sección Regional Neotropical de la Organización Internacional de Control Biológico (OICB). 24-28 Mayo 2004, Havana (Cuba). Abstracts, p. CS.4.7.

8. Mujica, N. (2004). IPM for the Leaf Miner Fly (LMF). Activity Report, period January 1, 2004- December 31, 2004. International Potato Center, 17 p

*9. Weintraub, P.G. and Mujica, N. (Submitted) Systemic Effects of a Spinosad Insecticide on *Liriomyza huidobrensis* Larvae. Phytoparasitica

*indicates joint Peruvian and Israeli authorship

V. Project Productivity

After the project proposal was written and submitted, work continued in both countries. Between 1997 and 2001, when the project was funded, a number of manuscripts were published by the Israeli author, directly relating to the proposed work (Weintraub, 1998-2001). Additionally, during that time, in Israel the leafminer became effectively controlled by a guild of native parasitoids. While it was observed by growers and researchers that the leafminer was causing less damage from 1998 onwards, the cause was not investigated. As a result of this project, a guild of parasitoids was identified but because the leafminer was no longer considered a serious pest, as it was at the time the proposal was written, the project goals on the Israeli side suffered. When there are no, or very, very low field populations, research on insecticide efficacy is equivocal – statistical results can not be obtained and publication is impossible.

On the Peruvian side, the project accomplished all of the stated goals, and then some. As was mentioned in the Executive Summary, parasitoids could not be sent to foreign experts for taxonomic identification/validation. Therefore, Norma Mujica was trained, and gained sufficient expertise to identify most of the Peruvian species, although

validation by an expert, such as John LaSalle (CSIRO) should yet be done. Additionally, the proposal to control the leafminer by the introduction of foreign predators parasitoids could not be considered.

> "In the third year, the most effective parasitoid(s) and predators, as determined by the previous years' work, will be released or augmented in the environment. *This may include introduction of non-native parasitoid and predator species.* Monitoring will continue as previously described to evaluate these effects. Carefully timed insecticide applications will be made as determined by the necessity of control."

VI Future Work

Our work will be approach to research the use of beneficial organisms and botanical insecticides to control leafminer fly. Some studies involving botanical extracts like chinaberry tree (*Melia azedarach* L.,Meliaceae), *Urginea maritime* L. (Liliaceae) (Civelek and Weintraub, 2004), *Euphorbia myrsinites* L. (Euphorbiaceae) and neem (*Azadirachta indica* (Meliaceae) have investigated the effect against *Liriomyza* species. In the Cañete valley, farmers are using aqueous extract of *Sapindus saponaria* (Sapindaceae) to reduce LMF population in snow pea fields, but the insecticide effect of this extract on LMF population is unknown. On the other hand, among the many fungal insect pathogens encountered in nature, *Paecilomyces fumosoroseus*, is considered a very promising biological control agent due to its extensive host range which includes insects in over 25 different families. In 2004, we recovered two strains of *P. fumosoroseus* from LMF adult and are maintained in CIP entomopathogen collection. Pathogenicity and effectiveness against LMF will be evaluated under laboratory and field conditions. The use of theses beneficial organisms and botanical insecticides can help reduce the levels of leaf miner fly populations and also contributing to reduce the use of insecticides

VII Literature Cited:

Alonso-Amelot, M.E., Avila, J.L., Otero, L.D., Mora, F., Wolff, B. (1994) A new bioassay for testing plant extracts ad pure compounds using red flour beetle *Tribolium castaneum* Herbst. J. Chem. Ecol. 20:1161-1177

Blanchard, E. (1926) A dipterous leaf-miner on Cineraria, new to science. *Rev. Soc. Entomol. Argent.* 1:10-11.

Chavez, G.L. and Raman K.V. (1987) Evaluation of trapping and trap types to reduce damage to potatoes by the leafminer, Liriomyza huidobrensis (Dioptera, Agromyzidae) *Insect Sci. Appl.* 8:369-372.

Civelek, H.S. and Weintraub, P.G. (2004) Effects of two plant extracts on larval serpentine leafminers, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), in tomatoes *J. Econ. Entomol.* 97:1581-1586.

van der Linden, A. (1991) Biological control of the leafminer Liriomyza huidobrensis (Blanchard) in Dutch glasshouse tomatoes. *Meded. Fac. Landbouww et. Rijksuniv. Gent* 56:265-271.

Weintraub, P.G. and Horowitz, A.R. (1995) The newest leafminer pest in Israel. *Liriomyza huidobrensis*. *Phytoparasitica* 23:177-184.

Weintraub, P.G. and Horowitz A.R. (1998) Effects of translaminar versus conventional insecticides on *Liriomyza huidobrensis* (Blannchard) (Diptera: Agromyzidae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) populations in celery. *J. Econ. Entomol.* 91: 1180-1185.

Weintraub, P.G. (1999) Effects of cyromazine and abamectin on the leafminer, *Liriomyza huidobrensis* and its parasitoid, *Diglyphus isaea* in celery *Ann. Appl. Biol.* 135: 547-554.

Weintraub, P.G. (2001) Effects of cyromazine and abamectin on the leafminer, *Liriomyza huidobrensis* and its parasitoid, *Diglyphus isaea* in potatoes. *Crop Protect.* 20: 207-213.

Weintraub, P.G. (2001) Changes in the dynamics of the leafminer, *Liriomyza huidobrensis*, in Israeli potato fields. *Intern'l J. of Pest Manage.* 47: 95-102

APPENDIX

Publications

Mujica, N. Espinoza, J. Lagnaoui, A. (2003). Integracion de clones resistentes e insecticides selectivos en el manejo de la mosca minadora *Liriomyza huidobrensis*. Abstracts of XLV Congress of the Entomological Society of Peru; 1-5 December 2003. Ayacucho (Peru). p. 161.

El ensayo se realizó entre los meses de Julio y Noviembre del 2002 en el valle de Canete Se condujeron parcelas con y sin aplicación de insecticida selectivos para evaluar la resistencia al daño de la mosca minadora en 11 genotipos de papa. Los tratamientos incluyeron 5 clones resistentes (382150.16, 385524.9, 393280.57, 97035.3 y 385561.12), una variedad tolerante (Maria tambeña), un clon susceptible (E86.011) y 5 variedades susceptibles (Canchan, Maria Reiche, Unica y Desiree). A los 90 dias despues de la siembra el nivel de daño foliar fue menor en los clones resistentes de papa que en las variedades insecticidas. Las variedades comerciales registraron un porcentaje de daño foliar de 41.3% y de 81.7% en parcelas con y sin insecticida respectivamente. Desiree presentó el mayor nivel de daño con 49% en parcelas aplicadas y 100% en parcelas sin aplicar. En los clones resistentes se observó un promedio de 18.3% de daño foliar en parcelas protegidas y de 32.8% en parcelas sin proteccion. Los clones 385561.12 y 393280.57 mostraron el menor porcentaje de daño foliar en parcelas tratadas (16%) y parcelas no tratadas (19 y 20%). Los clones 385524.9 y 393280.57 presentaron el menor nivel de infestación larval en parcelas con insecticidas (0.6 y 0.9 larvas/foliolo respectivamente) y sin insecticidas (0.8 y 1.5 9 larvas/foliolo respectivamente). El nivel de parasitismo se vio afectado por la aplicación de insecticidas. A la cosecha, la significativa para las variedades comerciales y no significativas para los clones resistentes. La integración de insecticidas selectivos con variedades y clones de papa con resistencia a mosca minadora, posibilita un manejo mas eficiente de la plaga bajo condiciones de campo.

Zevallos, R., Mujica, N. and P. Weintraub (2003). Efecto de la temperatura en la oviposicion, alimentacion y longevidad de *Liriomyza huidobrensis* (Diptera Agromyzidae). Abstracts of XLV Congress of the Entomological Society of Peru. 1-5 December 2003, Ayacucho (Peru). p. 159.

El presente trabajo se desarrolló para determinar la temperatura mas adecuada para la crianza masiva de la mosca minadora *L. huidobrensis*. Se evaluó el efecto de cinco temperaturas constantes (15, 18, 20, 22 y 26C) en la oviposición, alimentación y longevidad de la mosca minadora sobre haba, *Vicia faba*, como planta hospedera. Se observó una correlación negativa significtiva entre longevidad de la hembra y las temperaturas estudiadas. El número total de huevos/hembra fue similar a 20 (112.2) y 22 (111.2) y significativamente menor a 15 (72.7) y 26C (88.6). La mayor oviposición (169.2 huevos/hembra) y alimentación (3081.4 picaduras/hembra) ocurrió a 18C, registrándose esta temperatura como la mejor opción para mantener una crianza masiva de la mosca minadora en el laboratorio.

Mujica, N., Leiva N., Prudencio C., and O. Ortiz (2003). Efecto de insecticides sistémicos y translaminares sobre *Liriomyza huidobrensis* (Diptera: Agromyzidae) y sus hymenoptera parasitoids. Abstracts of XLV Congress of the Entomological Society of Peru; 1-5 December 2003, Ayacucho (Peru)., p. 138

Ensayos en campos experimentales fueron conducidos para evaluar el efecto de los insecticidas ciromazina (Trigard 75PS), abamectina (Vertimec 1.8CE), chlorfluazuron (Atabron 5CE), cartap (Padan 50PS), spinosad (Tracer 120SC) e imidacloprid (Confidor 350SC), sobre la población larval de la mosca minadora y sus hymenopteros parasitoides. A los 16 días después de la aplicación, ciromazina, abamectina y spinosad fueron los insecticidas más eficientes en el control de larvas de mosca minadora, con promedios de 0.4, 1.6 y 1.3 larvas/foliolo respectivamente. No se observaron diferencias significativas en el número de larvas/foliolo entre chlorfluazuron (6.0), cartap (4.5), imidacloprid (4.3) y el testigo absoluto (5.0). Ciromazina mostró una residualidad de 20 días. Se registraron 8 especies de parasitoides de las familias Pteromalidae (1), Eulophidae (5), Cynipidae (1) y Braconidae (1). *Halticoptera arduine* (Hymenoptera: Pteromalidae) fue la especie predominante antes y después de la aplicación de los tratamientos. Solo ciromazina redujo significativamente el número de individuos por especie y el número de especies de parasitoides, hasta 20 días despues de la aplicación. En el resto de insecticidas, las poblaciones de parasitoides lograron recuperarse a los 12 días después de la aplicación

Mujica, N., Weintraub, P., Hualla, V. and C. Prudencio (2004). Distribución y diversidad de la mosca minadora *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae) en hospederos cultivados en la costa del Peru. Abstracts of XLVI Congress of the Entomological Society of Peru; 7-11 November 2004, Arequipa (Peru). p. 61

La mosca minadora *L. huidobrensis* es una plaga principal que infesta una gran variedad de cultivos y sus enemigos naturales, en especial los parasitoides, constituyen una fuente potencial de agentes de control biológico que necesita ser estudiada. El presente trabajo buscó generar información sobre la distribución, diversidad y predominancia de las especies de hymenópteros parasitoides de mosca minadora en hospederos cultivados. La prospección se realizó entre los meses de Julio y Agosto del 2003 en 48 localidades de la costa peruana, desde Tumbes hasta Tacna. En cada localidad se colectó follaje infestado con mosca minadora, que luego fue mantenido en crianza hasta la emergencia de los adultos del minador y de sus parasitoides. Los especimenes fueron identificados al nivel de género o especie por medio de claves taxonómicas y por comparacion con una colección referencial. Se identificaron 42 cultivos hospederos de Agromyzidae, siendo 22 de ellos infestados por *L.huidobrensis*. Se recuperaron 17 especies de parasitoides pertenecientes a las familias Eulophidae (11 spp.), Braconidae (3 spp.), Eucolidae (1 spp.), Elachertinae (1 spp.) y Pteromalidae (1 spp.). La familia Pteromalidae fue dominante en las localidades costeras, con excepción de Tumbes y Tacna donde los Eulophidae fueron más abundantes. *Halticoptera arduine*, *Diglyphus websteri*, *D. begini*, *Ganaspidium* sp. y *Chrysonotomyia* sp fueron registrados en todos los departamentos de la costa peruana. *Chrysocharis flacilla* fue dominante en Tacna y *Ch. brethesi* en Tumbes. *H. arduine* representó el 51% de los especimenes y fue recuperada de puparios de las 22 especies cultivadas

Mujica, N. and A. Lagnaoui (2004). Efecto de la densidad de adultos de *Halticoptera arduine* (Hymenoptera: Pteromalidae) en el parasitismo larval en *Liriomyza huidobrensis* (Diptera: Agromyzidae). III Congreso Latinoamericano de la Sección Regional Neotropical de la Organización Internacional de Control Biológico (OICB), 24-28 Mayo 2004, Havana (Cuba). Abstracts, p. CS.4.7.

En el desarrollo de la crianza masiva del parasitoide larvo-pupal *H. arduine*, se probaron tres densidades de parasitación en condiciones de invernadero, usando *L. huidobrensis* como insecto hospedero y frijol (*Phaseolus vulgaris*) como planta hospedera. Las unidades de infestación consistieron en plántulas de frijol con hojas cotiledonales desarrolladas, las cuales fueron expuestas a adultos de *L. huidobrensis* de 4 días de edad, para oviposición por 6 horas, lo cual produjo un promedio de 15 larvas/hoja cotiledonal. Las unidades de parasitación contuvieron cuatro hojas cotiledonales con larvas entre segundo y tercer estadio larval, las que fueron expuestas a las densidades de una, dos y tres parejas de *H. Arduine*. Las hojas fueron cambiadas diariamente por un periodo de 7 días y en cada una de ellas luego se contabilizó el número de adultos de mosca minadora, parasitoides, puparios sin abrir y larvas no desarrolladas. La densidad de dos parejas/unidad de parasitación tuvo una producción total de 77.7 parasitoides, diferenciándose significativamente de los otros tratamientos. El mayor valor de parasitismo fue de 80.9% a la densidad de dos parejas/unidad de parasitación, con un pico de parasitismo de 92.3% al tercer día de exposición. La oviposición hembra de parasitoide se vió afectada por la densidad, registrándose una producción mínima de 12.7 parasitoides/hembra a la densidad de tres parejas/unidad de parasitación, siendo este resultado significativamente diferente con los otros tratamientos. Asimismo, la mayor densidad de parasitoides, incrementó la competencia intraespecifica, observándose una mortalidad de 30.8% de larvas en la densidad de 3 parejas/unidad de parasitación. Bajo condiciones de invernadero, la densidad de dos parejas/unidad de parasitacion resulto la más eficiente para fines de crianza masiva del parasitoide.

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Systemic Effects of a Spinosad Insecticide on *Liriomyza huidobrensis* Larvae

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In an effort to expand the spectrum of larvicides effective against *Liriomyza huidobrensis* (Blanchard), the effect of spinosad was studied on the development of the leafminer under laboratory conditions. Bean plants infested with various leafminer stages (egg through third instar) were treated by dipping leaves in 24 ppm, 48 ppm or 96 ppm a.i. spinosad or by drenching the soil of plants with 200 ml of water containing 12 mg, 24 mg or 48 mg a.i. spinosad. In general, leaf dipping was more efficacious and adverse effects were observed sooner than with soil drench. All concentrations of spinosad significantly reduced ($P < 0.001$) the number of adults except leaf dip of third instar; only the highest concentration caused significant reduction of pupae and adults. Spinosad would be a valuable insecticide for control of the pea leafminer, *L. huidobrensis*

KEY WORDS: *Liriomyza huidobrensis*; spinosad; systemic effects by soil drenching, leaf dipping.

The pea leafminer, *Liriomyza huidobrensis* (Blanchard), originated from South America (1) and is a serious pest of flower and vegetable crops in greenhouses and open fields in many parts of the world. It appeared in Israel in 1992 and adults are refractory to insecticides (12). Both the larvae and adults cause damage; the larvae primarily mine the spongy mesophyll, where chloroplasts are located (7), and the adult females puncture both the upper and lower leaf surfaces to feed and lay eggs. This results in reduction of both plant vigor and yield, and in cosmetic damage to leaves and stems.

There are currently no effective adulticides, and few effective larvicides - primarily, abamectin and cyromazine (13, 14) - against this pest; moreover, exclusive use of one of these larvicides could quickly generate resistance. Botanical insecticides derived from the actinomycete, *Saccharoolyhspora spinosa*, have been shown to be effective against lepidopteran larvae (5) stored product beetles and psocids (6), thrips (4) and tephritid flies (9). Spinosad has been used on a few agromyzid leafminers, *Chromatomyia horticola* (Goureau) (10), *Liriomyza chinensis* Kato (2) and *Liriomyza trifolii* (Burgess) (3). The purpose of this research was to evaluate the effect of systemic

(soil drench) and leaf dip applications of spinosad on the development of immature stages of *L. huidobrensis* to adult flies.

Bulgarian variety beans (*Phaeolus vulgaris*) were seeded in 10-cm-diam pots (4-6 per pot) with holes in the bottom, in soil consisting of equal parts peat moss, vermiculite and sand. Plants were grown at 23 ± 3° C, with a photoperiod of 14:10 (L:D), until two true leaves were fully expanded, unless otherwise stated. In all trials, plants were placed for ca. 3 h in a cage with a small number of adults from the stock *L. huidobrensis* colony. The short oviposition access period allowed for synchronous egg hatch and subsequent larval development. After oviposition, plants were removed and maintained in a growth chamber at 23° C and a photoperiod of 14:10 (L:D). In trials involving egg treatment, eggs were counted immediately after pots were removed from the stock colony of adults. In trials involving treatment of the three stages of larvae, the number was counted immediately before treatment by using a stereo-microscope with sub-stage lighting.

Spinosad (Tracer, imported by Agrochem, Petach Tikva, Israel) was used in all bioassays and was applied to the soil or leaves of bean plants. In the leaf-dip trials, soil was first watered to runoff, and then covered with thick layers of paper toweling to prevent spinosad dilutions from dripping off the leaves onto the soil, and thereby causing a systemic effect. The leaf dip consisted of water control, 24 ppm, 48 ppm or 96 ppm active ingredient (a.i.) spinosad. Leaves were dipped for 10 sec. For the drench, plants were watered 2 days before spinosad application. Soil was drenched with either 200 ml water (control), or 200 ml containing 12 mg a.i., 24 mg a.i. or 48 mg a.i. spinosad. Plants with eggs, or first, second or third instar larval leafminers were treated. To avoid possible adverse effects from the larvae being crowded on the leaf and stressed because of inadequate food, only leaves with less than 10 eggs or larvae were used. The number of individuals surviving to the subsequent stage was counted until pupae emerged. Paper envelopes were fashioned around leaves at last third instar to catch falling prepupae and prevent them from falling onto the soil or being lost. Pupae were placed in Petri dishes to observe adult emergence. Each trial was repeated until a minimum of 70 individuals were treated per concentration and application regime. Percent survival data were transformed by arc-sin before analysis. Data were analyzed using two-way analysis of variance (ANOVA), and means were separated using Tukey's HSD quantile function, using JMP5.0.1a (11).

For the leaf dip (Figure 1, LE, L1, L2, L3), all three concentrations of spinosad were most efficacious when applied to the egg stage ($df = 3, 99$, $F = 290.0$, $P < 0.0001$). Death occurred primarily in the first instar; presumably shortly after emergence from the egg since observed tunnels were usually less than 3 mm long. Only with the lowest concentration did some first instar larvae survive to second instar. With treatments at successively older larval instars, the spinosad was slightly less efficacious; only the highest concentration was significantly efficacious when third instar larvae were treated ($df = 3, 117$, $F = 3.57$, $P = 0.016$). Results of the soil drench are shown in Figure 1(DE, D1, D2, D3). In general, larval response (mortality) to the spinosad soil drench was slower; this because the insecticide was being taken up by the plant and acted in a systemic fashion. When the soil of plants with leafminer eggs was drenched, 80-90% of the first instar larvae were killed with the two highest concentrations; however with the lowest concentration only at the third instar stage were > 95% of all treated larvae killed.

Treatment of the third instar larvae yielded significant results (df = 3, 73, F = 46.65, P < 0.0001); however in the lowest concentration ca 50% of the adults survived.

These treatment concentrations were chosen to cause a wide range of mortality in *L. huidobrensis*. Unfortunately, new insecticides are usually over-used by growers which causes insecticide resistance. Parrella and Keil (8) showed that some insecticides became ineffective against *L. trifolii* only 2 years after introduction. Ferguson (3) showed that populations of *L. trifolii* collected from ornamental greenhouses had up to a 1,192-fold resistance (LC_{50} 1,586 ppm, leaf dip) to spinosad as compared to a susceptible reference, but reverted relatively quickly (< 10 generations) to reference-strain susceptibility when reared without insecticide pressure. Spinosad is very effective against all larval stages of *L. huidobrensis* and, if used judiciously by alternating its use with other translaminar insecticides, will give reliable control for a long time.

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