

Dispersal of the pea leaf miner *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae): a field experiment

Dispersión del minador de hojas *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae): un experimento a campo

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ABSTRACT

Movement of herbivore insects within agroecosystems can ultimately determine the level of damage to crops. It was evaluated the dispersal of *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae) under field conditions. In addition, it was evaluated if body size was related to dispersal distance. Eight hundred individuals of *L. huidobrensis* were released in the central point of a series of concentric circles (6, 12, 24, 48, and 96 m in radius), where 117 yellow sticky traps were set to insect recapture. Circular Statistics were used to evaluate flight direction whereas hurdle models were applied to analyze dispersal probability in relation to distance. Five percent of the released individuals were recaptured, being 36 m the median distance of recapture. The distribution of recaptured insects was not random around the circles, but the preferential dispersal direction was not explained by wind direction. The incidence of recaptured *L. huidobrensis* of both sexes significantly decreased at increasing distances from the release point, but decayed faster for females. No effect was found of body size on the distance of recapture. The results suggest that *L. huidobrensis* dispersed mainly over short distances with males being capable of performing longer flights than females.

Keywords

body size • herbivore insect pest • movement • wind

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RESUMEN

El movimiento de insectos herbívoros dentro de agroecosistemas puede finalmente determinar el nivel de daño a los cultivos. Se evaluó experimentalmente la dispersión de *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae) a campo y se analizó si el tamaño corporal se relacionó con la distancia de dispersión. Se liberaron ochocientos individuos en el punto central de una serie de círculos concéntricos (6, 12, 24, 48 y 96 m de radio), donde se colocaron 117 trampas pegajosas amarillas para recapturar los insectos. Se utilizó estadística circular para evaluar la dirección del vuelo, y modelos "hurdle" para analizar la probabilidad de dispersión en relación a la distancia. Se recapturó el 5% de los individuos liberados siendo 36 m con la distancia mediana de recaptura. La distribución de insectos alrededor de los círculos no fue aleatoria, pero la dirección de dispersión preferencial no se explicó por la dirección del viento. La incidencia de *L. huidobrensis* de ambos sexos disminuyó significativamente al aumentar la distancia, pero decayó más rápidamente para hembras. El tamaño corporal no influyó la distancia de recaptura. Estos resultados sugieren que *L. huidobrensis* se dispersó principalmente a distancias cortas, siendo los machos capaces de desarrollar vuelos más largos que las hembras.

Palabras clave

tamaño corporal • insecto herbívoro plaga • movimiento • viento

INTRODUCTION

Dispersal is a key process in ecology affecting the dynamics and persistence of insect populations (6). The term 'dispersal' can be used to describe both the movements towards a breeding location which implies gene flow across space, and 'trivial' movements represented by short foraging flights (3). Both active (locomotory) and passive (wind) components can account for the dispersal of flying insects, with the relative contribution of each one varying among species (17, 23).

Active dispersal can be affected by both environmental factors and by the movement ability of individuals which in turn depends on certain factors such as age, stage, sex and body size (3).

Differential resource allocation between sexes contributes to sex-biased dispersal. In many insect species, female dispersal is limited due to investments of resources in egg production (3). Regarding body size, it is expected that larger individuals have a higher active dispersal rate than smaller ones (10, 13). It has been shown that relatively longer wings would increase acceleration capacity and improve the efficiency of prolonged flights (4). Passive dispersal by wind is likely to be important for minute insects (8) but in general little is known about how the dispersal of insects is influenced by wind in the landscape (12, 20).

In agro-ecosystems, the dispersal of herbivores can ultimately determine its spatial distribution and the level of damage to crops. Thus, understanding the dispersal process in insect pests might be helpful to improve management strategies (15).

The pea leaf miner, *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae), is a polyphagous species that causes serious damage to several horticultural crops (2, 19). Originally from South America, this species has expanded its range and invaded many regions of the world, frequently reaching pest status (26). Damage to plants is mainly caused by larvae which excavate tunnels consuming mesophyll cells leaving intact the upper and lower leaf surfaces. This species tends to be resistant to commonly used pesticides, thus different management strategies are applied to control it (25).

For trap cropping, for example, it is relevant to know the dispersal behavior of the target herbivore in order to maximize pest control by adequately locating trap plants in relation to main crop (7).

Studying the movement of insects in their natural habitat is essential for understanding their biology and behavior but sometimes represents a great challenge, especially for small flying insects like adult dipteran leaf miners. One alternative to study dispersal patterns in insects is the Eulerian approach which relies on physical or biological 'traps' at some particular points where dispersers are sampled (16).

Although there are a few studies on flight patterns of *Liriomyza* species (5, 11, 18, 28), none of them tested dispersal through a release and recapture field experiment.

The aim of the present study was twofold: i) to evaluate the mean direction and distance of movement of *L. huidobrensis* individuals, and ii) to examine if body size was related to dispersal distance.

MATERIAL AND METHODS

A release and recapture field experiment was performed in order to evaluate movement of *L. huidobrensis* individuals. The study was conducted in a 10-ha lawn field for commercial purposes. The field was placed in the outskirts of Córdoba city (31°16'58.5" S, 64°02'31.04" W), located in Córdoba province (Argentina), where no *L. huidobrensis* host plants were found after conducting a careful inspection of the field. A total of 800 unfed 1-2 days old individuals of *L. huidobrensis* reared on *Vicia faba* L. (broad bean) plants in the laboratory, were released in the experiment in a single release event.

Field collected *L. huidobrensis* adults were offered broad beans in the laboratory. Pots (28 cm x 15 cm, 9 cm deep) containing 8-10 broad beans (6-10 leaves each) were placed in a cage (glass, wood and voile, 30 cm side length) with 20 pairs for 4 h, at room temperature, for mating and oviposition. This procedure was carried out simultaneously in several cages to obtain a high number of adults.

To recapture the insects, 117 yellow plastic and opaque sticky traps were used (20 cm x 20 cm) which are known to be highly attractive for leaf miners (14). Traps were placed in the field in a design of concentric circles at five distances from the central point (6, 12, 24, 48, and 96 m in radius), where flies were released (figure 1a, page XXX).

The location of the releasing point was arbitrarily selected but any influence of this position on this results is unlikely given the spatial homogeneity of the lawn field. Traps were spaced out regularly every 9.5 to 10 m on each ring and placed at 50 cm above ground (from the base of the trap) as this is the height were *L. huidobrensis* usually disperses (14).

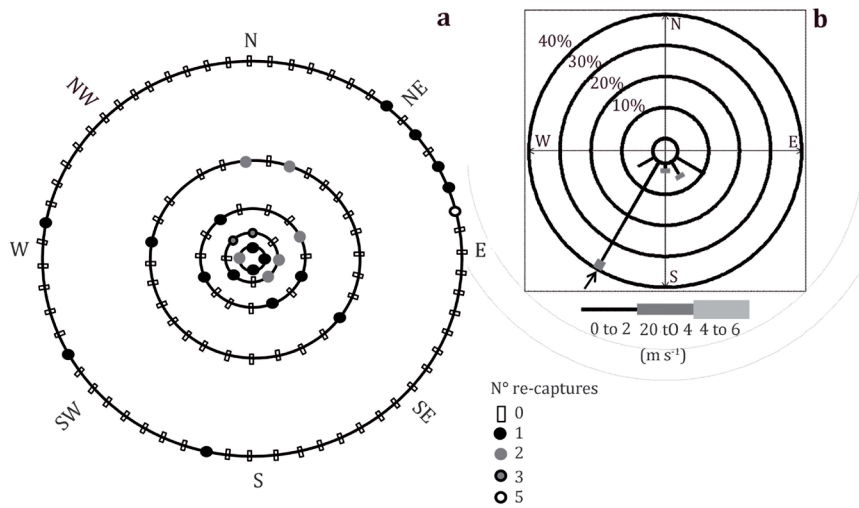


Figure 1. (a) Diagram of the field trial indicating the position of the 117 traps set at five distances (6, 12, 24, 48, and 96 m) from the central point where *Liriomyza huidobrensis* individuals were released. Different symbols indicate different number of re-captures (b) Circular histogram illustrating wind direction and speed during the experiment. Each ring represents the time proportion (%) of winds from different directions. The arrow indicates the prevailing wind direction.

Figura 1. (a) Diagrama del ensayo a campo que indica la posición de las 117 trampas establecidas a cinco distancias (6, 12, 24, 48 y 96 m) a partir del punto central donde se liberaron los individuos de *Liriomyza huidobrensis*. Diferentes símbolos indican diferente número de re-capturas (b) Histograma circular que ilustra las direcciones y velocidad del viento durante el experimento. Cada anillo representa la proporción de tiempo (%) en la que soplaron vientos desde diferentes direcciones. La flecha indica la dirección predominante del viento.

Leaf miners were released on July 16th 2014 at 11.30 am (after 15 min all flies leaved the box where they were transported) and traps were removed 24 h later and carried to the laboratory where they were inspected for *L. huidobrensis* adults.

The individuals collected were stored in tubes with 70% ethanol to posterior confirmation of its identity. For each individual, it was determined its sex and body size through the measurement of forewing length (mm).

The group of insects released had a female biased sex ratio (1.77:1 females

to males). A meteorological station Nexus (TFA) placed nearby the experiment automatically recorded wind speed and direction every 20 minutes.

Data analysis

Flight direction of *L. huidobrensis* was analyzed by means of Circular Statistics. A Rayleigh test for Randomness was performed to determine if the distribution of captures was distinguishable from random. To test whether the leaf miner re-capture direction was the same as the wind direction, a one-sample test,

analogous to a one-sample t-test for data on a linear scale, was conducted using wind direction as a pre-assigned angle to be tested against the leaf miner flight direction. Particularly, it was tested if insects flew downwind by subtracting 180° to the mean wind direction.

The angle (degrees) of each trap was measured in relation to the release point (through a drawing program) and then transformed into radians, which is the unit that Circular Statistics use. The analyses were performed by using the package "circular" (1) in R 3.2.2 (21).

To assess leaf miner dispersal in relation to distance from release we analyzed data ($N = 117$) with hurdle models which are a type of zero-inflated models (30) allowing to model the excess of zeros in the trap recaptured population (see Results, figure 1, page XXX). These models, which were performed separately for males and females, have two-parts whereby the first part is a binary outcome or incidence model, and the second part is a truncated count model (30).

In this models the explanatory variable for both parts was distance from release (m), the response variable was the number of recaptured flies per trap, and the assumed distributions were binomial for the incidence model and Poisson for the count model. Using this approach, it is possible to estimate the probability of recapture of each trap through the incidence model and then given *L. huidobrensis* individuals are present, estimate the relative mean number of individuals through the count model. Parameter estimates for distance were tested for significance using a Z- test. The hurdle models were performed using the package `pscl` (29) in R 3.2.2 (21).

Data on recapture distance were related to individual body size through

General Lineal Models separately by sex since females of *L. huidobrensis* are generally larger than males (24).

RESULTS

Five percent of the released individuals were recaptured in the traps. Although the liberated population was female biased there was a higher number of males recaptured ($n=29$) in relation to females ($n=14$). Considering the occupied traps ($n=28$), the mean distance of recapture of *L. huidobrensis* was of 46.71 m and the median 36 m.

The average wind direction during the experiment was 205° (*i.e.* direction from which wind blows, $N = 0^\circ$, $E = 90^\circ$, $S = 180^\circ$, $W = 270^\circ$), which indicates that the winds were preponderantly from the Southwest direction, with a wind speed of 0.4 m/s at the release event and with a mean of 0.65 m/s (range 0-2.4 m/s) during the whole duration of the experiment (figure 1b, page XXX).

The distribution of recaptured insects (figure 1 a, page XXX) was not random around the different circles according to the Rayleigh test ($z=0.28$, $P=0.03$), but their preferential direction was not explained by wind direction ($z=0.27$, $P=0.005$). In fact, the mean direction of insect recaptures was 188° which was upwind with respect to prevailing winds.

Hurdle models indicated that there was no effect of distance on the number of recaptured individuals (count model) of either sex (table 1, page XXX).

However, the probability of recaptured *L. huidobrensis* (incidence model) significantly decreased with distance for both males and females (table 1; figure 2, page XXX).

Table 1. Hurdle models for females and males of *Liriomyza huidobrensis* relating the distance from the release point to the probability that the species is present in a trap (incidence model) and the relative mean number of individuals in a trap (count model).

Tabla 1. Modelos "Hurdle" para hembras y machos de *Liriomyza huidobrensis* relacionando la distancia desde el punto de liberación y la probabilidad de que la especie esté presente en una trampa (modelo de incidencia) y el número medio relativo de individuos en una trampa (modelo de conteo).

Sex	Parts of the hurdle model	Estimate	Z	P
Females	Incidence model	-0.042	-3.228	0.001
	Count model	-0.019	-0.518	0.605
Males	Incidence model	-0.015	-2.112	0.034
	Count model	0.004	0.401	0.688

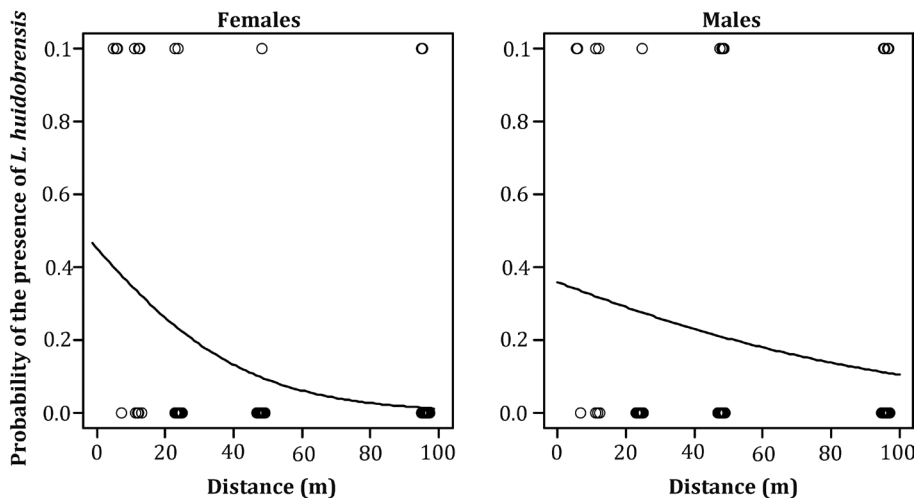


Figure 2. Relationship between the distance from the release point and the probability of the presence of *Liriomyza huidobrensis* females and males in each trap. There was a higher probability that males reached higher dispersal distances than females as it is shown by superimposed dots in the right panel.

Figura 2. Relación entre la distancia desde el punto de liberación y la probabilidad de presencia de hembras y machos de *Liriomyza huidobrensis* en cada trampa. Existe una mayor probabilidad de que los machos alcancen distancias de dispersión mayores que hembras tal como lo muestran los puntos super impuestos del panel derecho.

Although confidence intervals for distance parameters of females (95% CI: -0.072, -0.019) and males (95% CI: -0.03, -0.001) were superposed by 21%, the probability of recapture of each trap decayed faster with distance for females.

Regarding body size, there was not a significant relationship between wing length and dispersal distance of flies for either sex (**Females:** $F_{1,12}=0.001$, $P=0.97$; **Males:** $F_{1,27}=1.53$, $P=0.22$).

DISCUSSION

The results of this study show that the pea leaf miner dispersed mainly over short distances with males being capable of performing longer flights than females. Dispersal of flying insects from a specific point usually lead to a decline in density distribution at increasing distances (9, 22, 23).

Although could not be found a decreasing pattern for the abundance of flies recaptured in relation to distance, it was found that the higher probability to find *L. huidobrensis* was near to the release point, eventually falling with distance, and decaying faster for females. This result indicates that this sex was the most sensitive to longer distances, were a few individuals were re-captured. Increased captures of pea leaf miner males at long distances may be explained by high flight activity as they actively search for mates and food whereas females spend more time on leaves for oviposition (19).

Movement patterns could be affected by behavioral, morphological, physiological, genetic, and developmental attributes of focal individuals, as well as by environmental variables (16).

The higher number of males recaptured in relation to females, despite the released group was female biased, could be explained by differences in the level of attraction of the traps.

Martin *et al.* (2005), showed that translucent yellow sticky traps were more attractive for females than opaque traps, with no differences for males. Contrary to this expectations, it was could not detect an effect of the wing length of individuals with dispersal distance reached by the leaf miners of either sex, probably due to the low number of recaptures.

In addition, the low variability in wing length among released individuals of both sexes ($CV < 10\%$) could explain that result. Future experiments using a larger number of insects and low and high quality host plants would provide individuals with higher size differences (24).

Agromyzid flies are considered to be "moderate fliers" (27) because they tend to fly very short distances between host plants (28), but at the same time, they can move longer distances by wind dispersal (27). The results presented here showed that under the conditions of this experiment, wind had not a significant effect on *L. huidobrensis* dispersal suggesting that insects may be in part controlling their long distance movements (20). This is in agreement with the findings by Jones and Parrella (1986), for *L. trifolii*, a closely related species to *L. huidobrensis*. These authors showed that *L. trifolii* dispersed 26 m on average, but was capable to reach the furthest distance tested (102 m) in a greenhouse experiment free of wind.

In this study, wind speed at the moment of insect release was really low not exceeding 0.4 m/s and considering that mean direction of insect displacement was not downwind, it is unlikely that wind speed and direction did influence dispersal of the pea leaf miner in the trial.

However, as it was one release event it is uncertain what would happen with the dispersal pattern at higher wind speeds.

Aware that the models used to analyze *L. huidobrensis* dispersal are phenomenological, and thus fail to discriminate detailed components of the dispersal process (6), but the low rate of recaptures (Results, page XXX) did not allow performing mechanistic models

with reliable results. Nevertheless, these analyses allow describing and predicting general patterns of dispersal for the pea leaf miner which represents the first step to begin understanding the dispersal process of this pest in the field. Knowledge of the dispersal potential of *L. huidobrensis* is relevant by different reasons. At local scale, it could be useful in crop pest control, for the application of trap cropping strategy being the knowledge on adult dispersal important to decide trap plants location (7).

In addition, the use of yellow traps as monitoring tool for the pea leaf miner could be improved by adjusting space between traps (11). At landscape scale, knowing mean dispersal distance of *L. huidobrensis* could be important to predict its potential of spread to other fields (19).

On the other hand, considering that *L. huidobrensis* is invasive in numerous parts of the world (26), the knowledge of dispersal parameters is relevant since they can have an overwhelming effect on invasion speed (15). At the same time, the dispersal information could serve to model the future areas of colonization (in altitude and latitude) of the pest under

scenarios of global climate change (15). All in all, the information obtained in this field experiment could help to the future development of proper control strategies within an integrated pest management program and to develop forecasting systems to alert farmers about pest invasions and outbreaks (17).

CONCLUSIONS

In general the pea leaf miner showed a low dispersal capacity in a homogenous landscape. Nevertheless, the potential consequences of this behavior should be analyzed considering the generalist feeding habitat of the species. Future studies on the topic should incorporate different spatial configurations of the landscape in order to have bigger picture of the process. Obtaining field dispersal estimates, as those here showed is essential to understand the ecological and evolutionary bases of the dynamics and persistence of insect populations and would help to guide a proper design of management policies of this worldwide pest species.

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