

## MOVEMENT OF *SPODOPTERA FRUGIPERDA* ADULTS (LEPIDOPTERA: NOCTUIDAE) IN MAIZE IN BRAZIL

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### ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is one of the most important maize pests in the Americas and particularly in South America. With the adoption of genetically modified plants expressing *Bacillus thuringiensis* toxins for lepidopterous pest control, there is a need for establishing strategies to delay the development of insect resistance (e.g. refuge areas). Thus, information on target insects' dispersal is essential to improve pest management techniques. The objective of this work was to evaluate the dispersal capacity of *S. frugiperda* adults using mark-release-recapture techniques. Insects were marked using red oil-soluble dye in the larval artificial diet. Marked adults were released twice in each growing season (dry and wet) in southeastern Brazil in 2006 and 2007. Recapture of marked insects was performed using light and pheromone traps. Males are more attracted to light traps than females and the recapture rate was higher in the dry season than in the rainy season. The most adequate model to explain the relationship between flight distance and number of recaptured insects is  $y = a^2 / (1 + (2a^{1.8} + bx))^{2.6}$ , where  $y$  is the distance and  $x$  is the number captured. The maximum recapture distances were 806 m for males and 608 m for females. Therefore, strategies for establishment of refuges should take such distances into consideration.

Key Words: fall armyworm, release-recapture, insect dispersal, insect resistance

### RESUMO

A lagarta-do-cartucho, *Spodoptera frugiperda* (J. E. Smith), é uma das principais pragas de milho nas Américas e particularmente na América do Sul. Com a adoção de plantas geneticamente modificadas expressando toxinas de *Bacillus thuringiensis* para controle de lepidópteros pragas, há a necessidade do estabelecimento de estratégias para reduzir o desenvolvimento de resistência de insetos (ex.: áreas de refúgio). Assim, informação sobre a dispersão dos insetos alvos é essencial para aprimoramento das técnicas de manejo de pragas. O objetivo deste trabalho foi avaliar a capacidade de dispersão de adultos de *S. frugiperda* utilizando técnicas de marcação-liberação-recaptura. Os insetos foram marcados por meio de corante lipossolúvel incorporado à dieta artificial das larvas. Adultos marcados foram liberados duas vezes em cada safra (seca e chuvas) no sudeste do Brasil em 2006 e 2007. A recaptura dos insetos marcados foi obtida com armadilhas luminosas e armadilhas de feromônio. Machos foram mais atraídos para armadilhas luminosas do que as fêmeas e a maior recaptura ocorreu durante o período da seca. O modelo mais adequado para explicar a relação entre a distância de vôo e o número de indivíduos recapturados é dado por  $y = a^2 / (1 + (2a^{1.8} + bx))^{2.6}$ . Distâncias máximas de recaptura foram de 806 m para machos e 608 m para fêmeas. Assim, estratégias para estabelecimento de refúgio devem levar em consideração estas distâncias.

The Fall Armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a polyphagous species and is a member of a small herbivorous insect group (<10%) that utilizes more than 3 plant families as their natural hosts (Bernays & Graham 1988). This pest occurs from Argentina to the USA (Clark et al. 2007) and causes serious economic damage to maize growers in several Latin American countries. In maize, this pest is usually found in the whorl feeding on young leaves for 14 to 21 d, the period for larval

development (Melo & Silva 1987). It also feeds on the reproductive parts of maize plants (ears and tassels). Due to its cannibalistic behavior, only 1 or very few larvae can be found per plant, despite adults laying egg masses comprising hundreds of eggs (Sparks 1979). In tropical areas, up to 8 generations a year can occur in maize fields (Busato et al. 2005). Maize yield reduction can reach approximately 57% (Cruz et al. 1999). Currently, FAW has also increased its importance as a cotton pest in Brazil.

Biotechnological methods for pest control such as genetically modified plants have been employed for several years in various countries (Caprio 1998). However, the use of this technique can cause problems, including the progress of resistance by target Lepidoptera to plants that express insecticidal proteins. This is a major concern for growers and has been a topic for pest management researchers (Guse et al. 2002). Selection of resistant insects in a population may occur under the selection pressure of any insecticide (Peferoen 1997), including protein toxins expressed by transgenic plants.

In order to delay the evolution of resistance of insects to toxins of *Bacillus thuringiensis*, the utilization of refuge areas is a recommended alternative (e.g., Alstad & Andow 1995). The employment of such areas for the management of resistance is related to the insect's ecology, dispersal, and mating patterns. According to Gould (1998), refuges are distributed within and/or out of the transgenic field and allow the development of non-resistant insects, which will mate with resistant ones. Such areas aim to supply a large number of susceptible homozygous individuals (SS) to mate with susceptible heterozygotes (SR) or rare resistant homozygotes (RR), diluting the resistance alleles present in the population. Therefore, refuge areas may favor the occurrence of random mating between populations in treated and refuge areas (Caprio 1998). However, local (= non-migratory) insect movement should be understood in order to effectively design and organize refuge plantings. For moths, one common method to study local population movement is the mark-release-recapture technique (Hunt et al. 2001; Qureshi et al. 2006).

Although *S. frugiperda* is a significant economic pest and one of the targets for the development of genetically modified plants, studies on local movement of adults have been very limited. Thus, information on insect dispersal is essential not only for elaboration and implementation of pest management programs but also has considerable value for a better understanding of behavior and ecological interactions (Bullock et al. 2002). Therefore, the objective of this research was to evaluate the local movement of *S. frugiperda* adults by labeling, releasing, and recapturing insects in maize fields.

## MATERIALS AND METHODS

### Insects and Internal Labeling

*Spodoptera frugiperda* egg masses were obtained from a mass rearing facility maintained at Embrapa Maize and Sorghum Research Station, Sete Lagoas, MG, Brazil. Insects were then reared under laboratory conditions ( $25 \pm 3^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and 12 h photophase) on artificial bean-

based diet according to Parra (1999). For labeling insects internally, red dye Sudan Red 7B® (Aldrich Company Inc.) at 400 ppm concentration was added to artificial bean-based diet offered to larvae (Vilarinho et al. 2006). The dye was initially diluted in corn oil (4 g dye/40 mL oil) and added to the diet, adopting a proportion 4 mL oil solution per L during diet preparation (Ostlie et al. 1984). The diet was then poured in a plastic tray (40 cm  $\times$  25cm) and, after cooling, was sliced into cubes of approximately 4 cm<sup>3</sup> and individually distributed into 16-cell plastic trays. Second instar *S. frugiperda* were transferred to these cells, keeping 1 insect/cell because of cannibalism. The trays were sealed with plastic lids and kept in an acclimatized room ( $25 \pm 3^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and 12 h photophase) until pupal formation.

Internally labeled insects were collected upon reaching the pupal stage and taken to the field soon after the beginning of adult emergence. For adult release, pupae were mixed with vermiculite and placed in a wooden box (80 cm  $\times$  50 cm  $\times$  40 cm), keeping a 6 cm window to allow the outflow of recently emerged moths and to protect pupae and/or adults in the cages from rain or dew. The box was placed on a wooden base brushed with entomological glue (Tanglefoot®) to keep ants or other predators from reaching pupae.

### Black Light Traps and Pheromone Traps

Black light (BLB Sylvania®) traps were used for adult recapture (Hunt et al. 2001; Qureshi et al. 2006). During the recapture period, light traps were kept illuminated from sunset to sunrise. The electrical energy source was provided by a 40-ampere car battery connected to each light trap. Plastic bags were tied to the base of each light trap to hold caught moths. These bags were checked daily for 11 to 14 consecutive days (Showers et al. 1989; Simmons & Marti Jr. 1992). Right after sunrise, all traps were checked and collection bags retrieved. Batteries were recharged daily to assure power over night. Insects were killed with ethyl ether. The marked moths were identified and counted. Pheromone traps (ISCA Tecnologia, Iscalure®) were used to recapture *S. frugiperda* males marked during the dry season in 2006. Pheromone traps were checked on a daily basis. All insects were removed and checked for marking.

### Experimental Areas and Design

The experiments were carried out in commercial maize fields located in Pirajuba, MG ( $19^\circ 57'S$ ;  $48^\circ 45'W$ ) and in Jaboticabal, SP ( $21^\circ 15'S$ ;  $48^\circ 16'W$ ) (Table 1). Crops were cultivated according to conventional techniques (Fancelli & Dourado Neto 2000). Pesticides were not applied 1 week prior to the release or during the recap-

TABLE 1. INFORMATION ON EXPERIMENTAL AREAS USED TO RELEASE AND RECAPTURE MARKED *S. FRUGIPERDA* ADULTS.

Growing Season	Locality (Altitude)	Area (ha)	Previous Crop	Neighboring Crop Areas
2006 (Dry)	Pirajuba/MG (570 m)	21	Soybean	Sugarcane, sorghum and forest fragment
2006/2007 (Wet)	Jaboticabal/SP (595 m)	40	Soybean	Maize, rubber tree and sugarcane

ture period. The release of marked *S. frugiperda* adults occurred during different phases of corn development (Ritchie et al. 1997) (Table 2). Insects were released during the first study at Pirajuba on 12 Apr and 5 May 2006 when maize plants were in vegetative stages V3 and V6, respectively (Table 2).

Light traps were placed in 2 parallel lines (200 m apart) and distributed over the farming area (Fig. 1). The release site was located on the north end of the area because the predominant wind direction is Southwest to Northeast, and the emerged moths would fly upwind. Two pheromone traps were placed within the trapped area (600 and 700 m from the release site), but between the light trap lines, whereas 15 pheromone traps were distributed at random around the study area. These pheromone traps were placed 100-1000 m from the experimental area edge.

The second study was carried out during the 2006/2007 growing season (wet season) in Jaboticabal. Trap distribution was similar to that adopted for the dry season study in 2006, but 2 further traps were installed (a total of 9 light traps) in each line (Fig. 1). The distances from the point of release were 100, 141.4, 223.6, 312.2, 412.3, 509.9, 608.3, 707.1, and 806.2 m. The 2 furthest distances were used only during the 2006/2007 growing season. Eight pheromone traps were installed around the experimental area as in the previous study. Adults were released twice; the first release occurred on 20 Dec 2006 when maize plants were in the V8 stage and the second release was on 11 Jan 2007 when plants were in the tassel stage (VT) (Table 1).

#### Survey of Marked Egg Masses

Females internally labeled with Sudan Red 7B® dye produce marked egg masses which can be located on maize plants. Marked egg masses were surveyed on a daily basis during the release-recapture evaluation periods for both studies (dry season in 2006 and wet season in 2006/2007). Plants were selected at random by zigzag walking in the field. At least 200 plants were observed thoroughly for presence of red colored egg masses (Vilarinho et al. 2006). Whenever an egg mass was found, the distance from the release source and the plant was measured.

#### Statistical Analyses

Data were submitted to analysis of variance and separation of means by Tukey's test ( $P < 0.05$ ) in PROC GLM and PROC REG (SAS Institute 2004). Data transformation by  $\sqrt{x+5}$  was necessary due to lack of normality. The Simplex and Quasi-Newton methods were used to estimate the parameters of dispersal models, adopting the exponential decreasing model  $y = a^2 / (1 + (2a^{1.8} + bx))^{2.6}$  for the adjustment of recapture analyses of insects with the Statistica (Statsoft 6.0) program. This model was adapted from Qureshi et al. (2005, 2006) and the variables  $a$  and  $b$  are parameters of the exponential model that were estimated to explain the relationship between insects recaptured and flight distance. The model fitness was evaluated by predicted error, mean absolute error, and mean square error.

Percentages of the number of marked recaptured insects were calculated. The number of released insects was standardized to 10,000 indi-

TABLE 2. MARKED *S. FRUGIPERDA* ADULTS RELEASED DURING DIFFERENT MAIZE STAGES AND RECAPTURE PERIODS.

Growing Season / Year	Release Date	Number of Releases	Phenological *Stage	Number of insects released	Recapture Period (days)
2006 (Dry)	12 Apr 2006	1st	V3	10,600	14
	5 May 2006	2nd	V6	11,500	14
2006/2007 (Wet)	20 Dec 2006	1st	V8	10,400	11
	11 Jan 2007	2nd	Tasseling	8,400	11

\*V3 - usually 2 wk after emergence and collar of the 3-leaf visible; V6 - usually 3 wk after emergence, collar of the 6th leaf visible, tassel has already been initiated, and the lower leaves tend to tear off; V8 - usually 4 wk after emergence, collar of the 8th leaf visible; VT (tasseling) usually occurs 60 d (8.6 wk) after emergence and 2 or 3 d before silking.

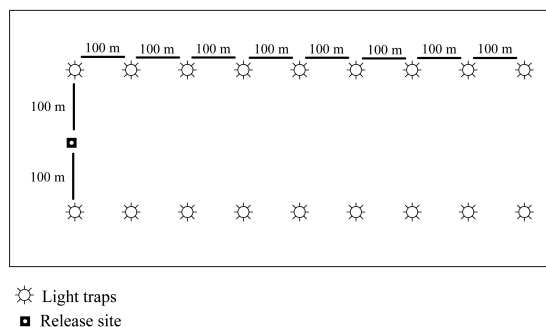


Fig. 1. Distribution of light traps in maize field during 2006/2007 wet season.

viduals for further comparisons. Mean number of recaptured insects was calculated for traps in the same distance throughout the recapturing period.

## RESULTS AND DISCUSSION

### Recapture of *S. frugiperda* by Light Traps

The mean number of adult *S. frugiperda* males and females recaptured was significantly higher in the dry growing season (2006) (males:  $F = 14.5$ ,  $df = 1, 56$ ,  $P = 0.0003$ ; females:  $F = 15.9$ ,  $df = 1, 56$ ,  $P = 0.0002$ ; total:  $F = 19.6$ ,  $df = 1, 56$ ,  $P = 0.0001$ ) than in the wet season (2006/2007) (Table 3). The total number of adults caught in light traps was 2.3 fold higher in the dry season. Thus, regardless of the release or study site, recapture was lower during the wet season, probably due to high frequency of rainfall during this season. In Mexico, Rojas et al. (2004) found a negative correlation between number of moths captured by pheromone traps and humidity. Thus, the prevalence of FAW during the dry season is usually higher. Dry season (2006) maize plants were underdeveloped in relation to plants grown during the wet season (2006/2007), because dry-season-grown maize is

rarely irrigated. Due to this underdevelopment they presented less leaf area and height, reducing obstacles for moth movement, as they typically move a few m from plant to plant when plants are at low height (ca. 1 m above the plant's maximal height) (Vilarinho, E.C., personal observation).

During the study period in the dry season (2006) there was no precipitation. This may have been a favorable factor for the higher number of recaptured insects relative to the 2006/2007 growing season (wet), during which it rained almost every night, possibly affecting the flight of the moths.

During the 2 releases in the 2006 and 2006/2007 growing seasons, female recapture was significantly higher after the first release ( $F = 22.58$ ,  $df = 1, 56$ ,  $P < 0.0001$ ), when plants were in younger vegetative stages, V3 and V8, respectively. Nevertheless, there was no significant difference between male recaptures ( $F = 0.49$ ,  $df = 1, 56$ ,  $P = 0.4887$ ). A comparison between the parallel lines of light traps showed no difference in insect catches during the 2006 and 2006/2007 growing seasons (males:  $F = 0.04$ ,  $df = 1, 56$ ,  $P = 0.8387$ ; females:  $F = 0.29$ ,  $df = 1, 56$ ,  $P = 0.5909$ ; total:  $F = 0.05$ ,  $df = 1, 56$ ,  $P = 0.8204$ ). Therefore, this is an indication that *S. frugiperda* movement occurred in a diffuse manner in all directions.

### Adult *S. frugiperda* Movement

During the 2 releases in the dry season (2006), only 0.09% of the released adults were recaptured. The highest percentages of recaptured adults occurred approximately 141 m from the release site. A total of 48.3% of all insects caught was observed in this range, which included traps located at 100 and 141.4 m. The percentage of insects recaptured in the traps located between approximately 220 and 610 m varied between 8.5% and 11.9%. In this range, traps were located at 5 different distances and caught 51.7% of the total recaptured insects. Moths were caught even at

TABLE 3. MEAN NUMBER OF ADULTS ( $\pm$ SE) OF *S. FRUGIPERDA* (MALES AND FEMALES) RECAPTURED BY LIGHT TRAPS THROUGHOUT THE RECAPTURE PERIOD IN MAIZE, DURING 2006 DRY SEASON AND 2006/2007 WET SEASON.

Group		Mean ( $\pm$ SE) <sup>a</sup>		
		Male	Female	Total
Season	Dry (2006)	4.74 $\pm$ 0.75 a	2.28 $\pm$ 0.59 a	7.02 $\pm$ 1.29 a
	Wet (2006/2007)	2.41 $\pm$ 0.43 b	0.56 $\pm$ 0.15 b	2.97 $\pm$ 0.51 b
Release	1st	3.52 $\pm$ 0.74 a	2.19 $\pm$ 0.52 a	5.71 $\pm$ 1.23 a
	2nd	3.35 $\pm$ 0.47 a	0.43 $\pm$ 0.16 b	3.78 $\pm$ 0.55 a
Rows of light traps	1	3.43 $\pm$ 0.54 a	1.40 $\pm$ 0.34 a	4.82 $\pm$ 0.76 a
	2	3.44 $\pm$ 0.69 a	1.22 $\pm$ 0.48 a	4.66 $\pm$ 1.14 a

<sup>a</sup>Means followed by a different letter are significantly different for each group within each column (Tukey's test,  $P < 0.05$ ).



the longest distances (509.9 m and 608.9 m) during this trial. There was a negative relationship between the number of insects caught and distance from the release site.

A separate analysis of the first and second release-recapture study indicated that adult dispersal over distance was similar, regardless of plant development stages (V3 and V6) (Fig. 2a). Regardless of the crop stage of development, the number of recaptured insects always decreased with the distance from the release site. During the first release, 166 insects were recaptured (1.66% of the total released), while in the second release, 45 were caught (0.45% of the total released). No precipitation occurred in the area of

study during the entire period of this recapture cycle.

The percentage of recaptured males was always higher than females (Fig. 2b, c), although the sex ratio of labeled insects at the time of release was ca. 1:1. A higher ratio of male recapture was also observed by Qureshi et al. (2006) for *Diatraea grandiosella* Dyar (Lepidoptera: Crambidae). Females were not recaptured at 312.2, 412.3, and 608.3 m away from the release site (Fig. 2c). In studies with *O. nubilalis*, Showers et al. (2001) observed that males can disperse over distances greater than 800 m in search of females; however, the highest percentage of recaptured males occurred at 200 m from the release point.

Within the internal area of light trap distribution in the field, 4 males were recaptured in pheromone traps, with 1 collected 600 m from the release site. No recapture of labeled individuals was observed in any of the other pheromone traps located outside of the maize field, possibly indicating that most of the insects remained in the field although the recapture rate was still low. It is also possible that the mortality rate of adults was high, although it was not assessed.

In the 2006/2007 wet season, *S. frugiperda*-labeled moths were recaptured in higher numbers 100 m from the release site; however, 47.4% of the insects were recaptured up to 223.6 m from the point of release. Adults were recaptured at all distances from the point of release, including the longest distance (806.2 m). More than 90% of *D. grandiosella* adults were recaptured in the range of 300 m from the point of release (Qureshi et al. 2006). In a similar study with *O. nubilalis*, Hunt et al. (2001) recaptured the majority of insects (70% to 98%) within 450 m from the site of release.

Only in the second release, when plants were at the tasseling stage (VT), were insects recaptured at all distances (Fig. 3a). In contrast, when corn plants were in the vegetative stage, insects were recaptured at shorter distances. The maximum distance of recapture of *S. frugiperda* during the first release-recapture was 608.3 m from the point of release (Fig. 3b). In the second release adults were caught by traps located 707 m (females) and 806 m (males) from the point of release (Fig. 3c). The number of adults recaptured was higher in the second release (68 adults; 0.65% of the total released) in relation to the first release (27 adults; 0.32% of the total). Only males were recaptured in all light traps after the second release (Fig. 3c). Females were recaptured at only 3 distances (100; 312.2 and 707.1 m) and at a much lower percentages than males (Fig. 3c). The recapture of males showed higher proportions and regularity relative to the females.

Two marked males were recaptured in only 1 pheromone trap located at 100 m from the point of

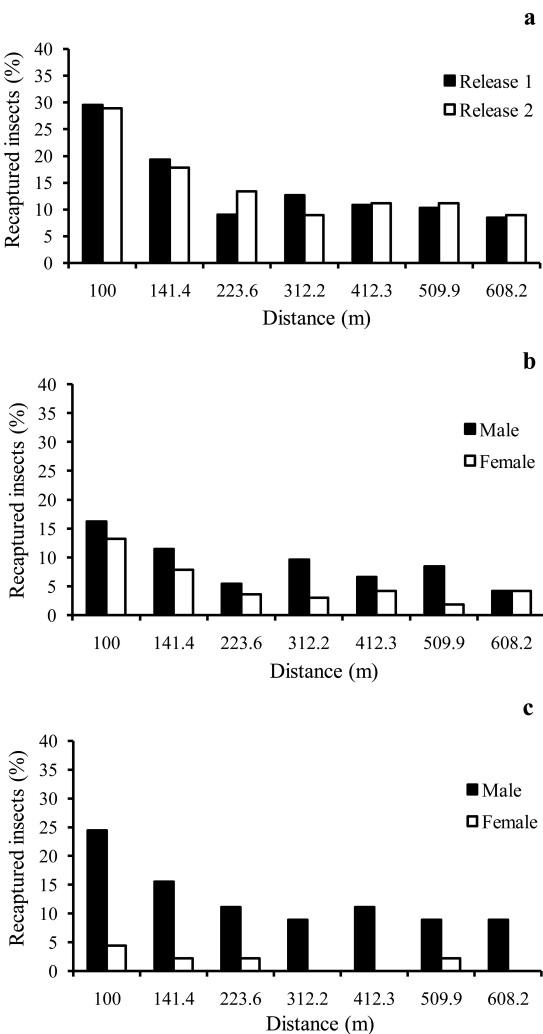


Fig. 2. Percentage of recaptured *S. frugiperda* adults (male + female) by light traps in the first and second releases (a), recaptured *S. frugiperda* adults in first (b) and second release (c) over distance from the release site (2006 dry season).

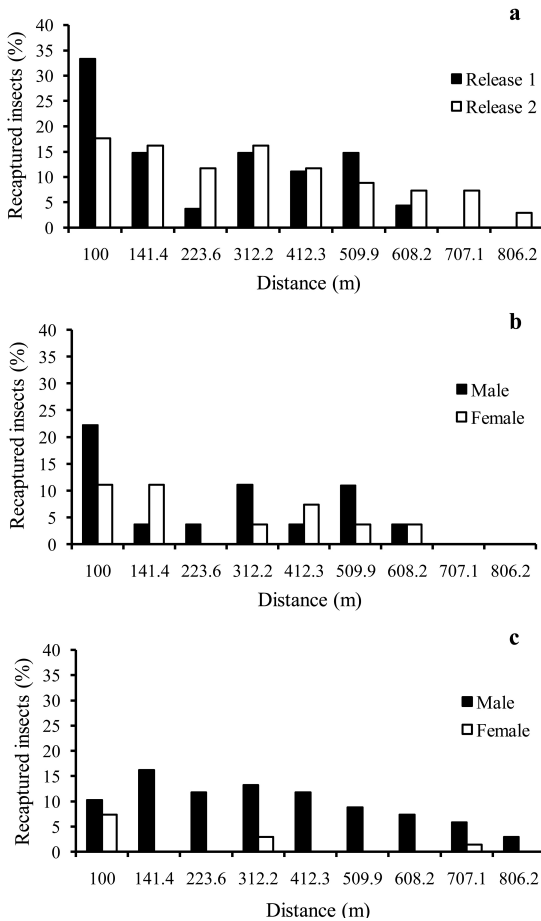


Fig. 3. Percentage of recaptured *S. frugiperda* adults (male + female) by light traps in the first and second releases (a), recaptured *S. frugiperda* adults in first (b) and second release (c) over distance from the release site (2007 wet season). The markers on the abscissa indicate the distance of the black light trap from the release site.

release. No recaptures occurred in any pheromone traps placed around the study field.

Survey of Labeled Egg Masses

The majority of egg masses labeled with the red dye found on plants during the 2006 dry season was located on the upper portion and adaxial side of either first, second, or third leaf below the flag leaf. The same pattern was observed for egg masses laid by naturally-occurring individuals. Marked egg masses had an average of 200 eggs, similar to the number of eggs normally recorded (Vilarinho et al. 2006). All egg masses found were fertilized because larval eclosion was observed.

During the 2006 dry season, higher percentage of marked and fertile egg masses occurred at 12 m

from the release site. Therefore, females were able to mate and lay eggs in the field in which they were released. Thus, despite the great number of insects released, intraspecific competition for mating and host did not appear to affect the population strongly. One marked egg mass of a mated moth at the longest distance of 608.3 m from the release site was recorded on the fourth day after adult emergence. During the 2006/2007 season there were marked egg masses found over a 12-m radius from the point of release.

Regression Analyses for *S. frugiperda* Recapture

The model  $y = a^2 / (1 + (2a^{1.8} + bx)^{(2.6)})$  explained the dependent relationship between flight distance and captured insects for both releases and growing seasons. This indicates that there was a reduction of recapture with distance from the release site ( $P < 0.03$ ) (Fig. 4a, d, g, j).

For females recaptured during the second release on both growing seasons and for males recaptured on the first release of the wet season, the parameters of the model were not significant ( $P > 0.05$ ). This occurred due to the very low number of insects recaptured. Thus the tendency of reduction in capture was not evident (Fig. 4f, h, l). On the remaining cases (males and females analyzed separately), the model explained the relationship between distance and number of insects ( $P < 0.05$ ) (Fig. 4b, c, e, k, i).

During the first release in the dry season (2006), the mean square error was the largest due to higher captures of both males and females at distances larger than 300 m. However, this was not observed in the other releases. For the remaining parameters, the results were similar in all conditions evaluated (Table 4).

During the 2006 dry season, the number of recaptures for both males and females after the first release decreased markedly up to 200 m from the release site (Fig. 4a-c). This trend was also observed for the second release (Fig. 4d-f). During the first release in the 2006/2007 wet season, the number of recaptured insects declined to 1-4 insects/trap from 141.4 m and beyond (Fig. 4g-i). Nevertheless, recapture of the total insects in the second release (Fig. 4j) and of males (Fig. 4k) demonstrated a gradual decrease of recapture along the distance ( $P < 0.05$ ). Problems to fit a model to data for females recaptured after the second release occurred because only 8 were recaptured in just 3 light traps (Fig. 4l), which fitted a negative model.

The recapture patterns were similar between growing seasons, although recapture of adults by light traps was higher during the dry season. There was a slight difference between plant stages (vegetative and reproductive), with recapture of adults higher during the vegetative stage (first release). Thus, during the dry season adults

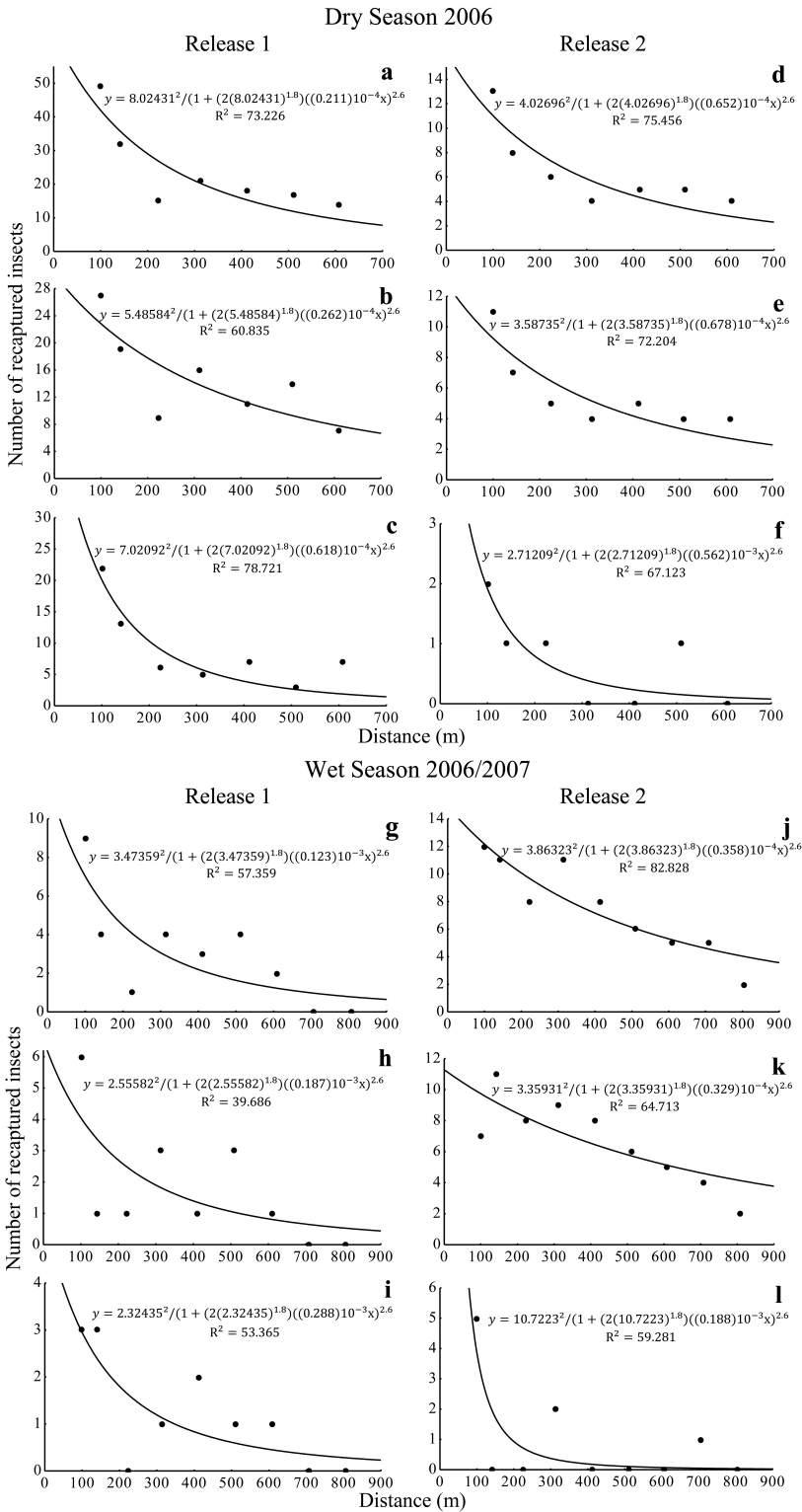


Fig. 4. Relationship of recaptured *S. frugiperda* adults (male + female) (a, d, g, j), male (b, e, h, k) and female (c, f, i, l) by light traps and distance from the release site during 2 growing seasons (dry and wet).

TABLE 4. FITNESS OF THE MODEL PARAMETERS.

Season	Release	Sex	Predicted Error (%)	Mean Absolute Error	Mean Square Error
2006 (Dry)	1st	Male	4.763	0.669	15.920
		Female	8.700	0.720	7.721
		Both	2.606	0.602	36.850
	2nd	Male	2.215	0.124	1.566
		Female	5.628	0.038	0.161
		Both	2.052	0.129	2.164
2006/2007 (Wet)	1st	Male	-0.661	-0.012	1.981
		Female	4.350	0.051	0.573
		Both	1.699	0.050	2.937
	2nd	Male	-0.778	-0.052	2.352
		Female	9.809	0.079	1.036
		Both	-0.368	-0.028	1.721

tended to stay in the corn field whereas during the wet season adults dispersed away from the field. In general, it seems males and females are behaving similarly; however, instead of searching for maize, males are searching for mates.

Overall, *S. frugiperda* adults moved as far as 806.2 m (the farthest location studied), although the majority was caught near the release site. It is likely that these insects move even further depending on the conditions at the location such as plant stage, oviposition sites, food resource availability, mate availability, and other factors. Thus, although its generalist behavior should be taken into consideration for refuge placement, refuge located about every 800 m for large maize fields should be suitable. This is similar to the range established for ECB in the United States (ILSI HESI 1998, U.S. EPA 2000). In Argentina, refuge should be planted in the center of areas longer than 1500 m, in order to maintain a maximum distance of 750 m between each other.

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