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Aggregation Behavior of *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae): Relationships Between Sites Chosen for Mating and Offspring Distribution

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Comportamento de Agregação de *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae): Relações Entre Locais Escolhidos Para o Acasalamento e a Distribuição da Progênie

RESUMO - Foi estudado o comportamento de agregação de *Phyllophaga cuyabana* (Moser) e investigada a hipótese de que a distribuição agrupada das larvas dessa praga no solo está relacionada aos sítios onde as fêmeas da geração anterior se agregaram para acasalamento. Observações de campo e experimentos de casa-de-vegetação no estado do Paraná, indicaram que: a) durante a revoada, fêmeas adultas de *P. cuyabana* selecionam as plantas mais conspícuas para pousar e atrair os machos; b) plantas altas, próximas de lavouras de soja, podem ser preferidas uma vez que os adultos não se movem para longe dos sítios de acasalamento antes de se enterrarem no solo para ovipositar; c) altas densidades de ovos e larvas ocorrem próximas aos locais de agregação; d) há uma correlação negativa entre a densidade larval no solo e a distância dos sítios de agregação de adultos.

PALAVRAS-CHAVE: *Glycine max*, Scarabaeoidea, praga de solo, distribuição espacial

ABSTRACT - The aggregation behavior of *Phyllophaga cuyabana* (Moser) was studied and the hypothesis that the cluster distribution of larvae of this pest into the soil is related to the sites where females from the previous generation had aggregated for mating was investigated. Field observations and greenhouse experiments carried out in Londrina County, State of Paraná, South Brazil, indicated that: a) during flight, adult females of *P. cuyabana* select plants, which are more conspicuous for landing and/or attracting males; b) tall plants, nearby soybean fields, might be preferred because female adults do not move far from the mating sites before digging themselves into the soil to lay eggs; c) high density of eggs and larvae occurs close to the adult aggregation sites. There is a negative correlation between larval density in the soil and distance from adult aggregation sites.

KEY WORDS: *Glycine max*, Scarabaeoidea, soil pest, spatial distribution

In the last decade, a complex of native white grubs has been reported as new pests damaging soybean crops in Brazil. Among the most common species, *Phyllophaga cuyabana* (Moser) predominates in the West Central Region of Paraná State, where, in some specific areas, it reaches the status of major soybean pest, feeding mainly on secondary roots (Oliveira *et al.* 1992, Santos 1992). Species of the *Phyllophaga* genus have also been reported as pests in many other countries where soybean is cultivated (Turnipseed & Kogan 1976, Eastman 1980, Lentz 1985). Chemical control of *P. cuyabana* has been ineffective and its biological control is discouraging, particularly because larvae live deep into the soil and reproductive adults spend only a short time above ground. A better understanding of the species biology and ecology can offer new insights for the management of this pest.

Field observations as well as preliminary tests in greenhouse indicate that cluster distribution is a common feature either for larval population in the soil and/or for reproductive adults swarming above ground. Evidence of *P. cuyabana* larvae occurrence in soybean fields is the incidence of large patches of dead or slowly growing plants with yellow leaves (Oliveira *et al.* 1992). This pattern of damage indicates that larvae are aggregated in the soil. Adults also aggregate for reproduction. During November and early December, adults leave the soil, flying at twilight and concentrating at special sites to mate. Females release pheromone soon after landing, and both mating adults return to the soil soon after copulation, which occurs only once at each night (Santos 1992). Both males and females leave the soil, flying to mate many times during the mating season (Oliveira *et al.* 1996). Although this is not a daily activity for the same individual since the majority of adults fly out on alternate nights (Oliveira 1997). After initiating copulation, the male would be attached to the female, maintaining a perpendicular position in relation to it. During copulation, the pairs remain practically immobile. After copulation, which in the field takes about 84 min. and occurs only once each night, males and females remain static on the plant for up to 30 min. (Oliveira *et al.* 1996, Oliveira & Garcia 2003).

Agricultural technicians and farmers have reported that during the flight season, adults usually land on the upper parts of soybean and/or other plants, and seem to be attracted to the most conspicuous substrates in the field. Large concentrations of mating males and females were observed either on tall plants, isolated trees, or on a line of trees (acting as windbreaks) in soybean fields. Aggregation of adults is also very common on maize plants growing closely to soybean fields. At the time of adult flight, maize is usually about 1 m high. Even under greenhouse and/or cage assays conditions, females leave the soil to searched for elevated sites on which to land (Oliveira 1997). In these cases, copulation usually occurs on the ceiling and/or upper parts and walls of the cages or greenhouse. After mating, the adults tend to walk a short distance before digging themselves into the soil.

Based on these observations, the hypothesis that the distribution of the offspring in the soil is directly related to the sites chosen for mating by adults from the previous generation was raised. The objective of the present study is to determine if more conspicuous sites to which adults are attracted to mate are related to sites chosen by females for feeding, oviposition, and establishment of the next generation.

Materials and Methods

This field study and the collection of insects for greenhouse experiments were carried out in commercial soybean fields in Juranda County, in the West-Central Region of Paraná State, South Brazil (24º25' South; 52º48' West).

Influence of Plant Height on Mating Site Choices in the Field. During two different reproductive seasons, mated and non-mated adults were counted, and the height and nature of the substrates on which they had landed were registered. On November 24th, 1994, an evaluation was made on a 1.60 m high x 1.80 m wide shrub patch, within a soybean field. The existing vegetation was divided into two different halves of vegetation height levels, one from 0 cm to 80 cm and the other above 80 cm, to facilitate insect counting. These counts were randomly taken into four 1m wide plots. In 1994, only females were counted. On November 3rd, 1995, adults (males and females) landing on three rows of about 3m high trees were counted. Again, the vegetation was divided in two sectors, 0 to 1.20 m and 1.20 to 2.5 m. The maximum height considered was lower than the height of the trees (since these had irregular sizes) so that females and males could be easily observed. Countings of all mating and non-mating males and females of *P. cuyabana* was carried out in four 3m wide plots, randomly taken from each row. The composition of the vegetation was not homogeneous. Mainly weedy herbs and shrubs composed the lower sector, while the branches of the windbreak trees represented the upper section.

The total number of plots evaluated in each year was determined according to the number of vegetation lines associated to beetle aggregation. Males and females were differentiated by the size of the antennae, which are larger in the males. In 1995, the evaluations were made in the four plots at the same time by four different observers, and each person repeated the counting in the other four plots. In both years, the proportion of males and females (out of the total counted) was determined for each observation night. The F test was used to compare the number of adults as well as the proportion of them that were mating on the upper and lower parts of the vegetation. In both years, the beetle counting began as soon the flights, "calling" and courtship behavior (Oliveira & Garcia 2003) ceased, about 40 min. after sunset, when all adults had landed on the plants. The adults were observed until the end of mating period.

Influence of Plant Height on Sites Chosen for Mating and Egg-Laying in the Greenhouse. Comparing the performance of *P. cuyabana* on maize and soybean, when the insect was confined to a single host or allowed to choose between both plant species Oliveira (1997) found no significant difference between the two hosts, regarding larval development, attraction of adults or number of eggs laid next to each plant species. In the field, however, aggregations of *P. cuyabana* adults were frequently found on the border of cornfields sowed in September next to soybean fields sowed in the second half of October. This aggregation phenomenon was also common with other taller plant species found next to soybean fields. A completely randomized experimental design with two treatments and 10 replications was used in this experiment. There was one set of treatments and one set of controls; treatments and controls differed only in plant height. Each replication was composed of an 80cm x 80cm x 50cm cement box, containing soil, which was then covered by a 1m tall screen-made cage. In each box there were three soybean (*Glycine max*) plants on one side and three maize (*Zea mays*) plants on the other side. Artificial infestation was accomplished by placing 15 adult pairs of *P. cuyabana* in the centre of each cage. Maize was sown before soybean and, at infestation time, the maize plants were at least 15 cm to 20 cm higher than the soybean plants in the treatment cages. The same methodology, using 10 cages with soybean and maize plants at a similar height and age, was used in the control boxes. Results from both test and control replicates were evaluated by carefully inspecting the soil of each cage, counting adults and eggs, which were manually separated from the soil with the aid of a spoon, 10 days after infestation. The number of feeding marks on soybean and corn leaves (caused by females feeding) was recorded. Based on preliminary tests, a minimal ingested area of 0.25 cm^2 was considered as a single feeding mark. Feeding marks larger than this minimal value were considered as two or more marks, according to their estimated areas. The "*t*" test was used to compare the number of feeding marks and egg-laying near the different plant species. Another previous test, using a similar methodology, was performed comparing soybean plants with different heights (one 20 cm taller than the other) and soybean plants with same height; in these tests only the number of eggs and adults were evaluated. However, the test in which the maize plants (taller) were compared with soybean plants (lower) corresponds to the situation most commonly found at the field study sites.

Mating Sites and Distribution of Adults and Eggs in the Soybean Field. In early November 1994, 1,200 mostly mating adults were collected from trees aligned in a 2 m wide row of wind breaking plants in a soybean field. Adults were collected manually, maintained in plastic bags during collection, marked with a paper corrector fluid "error/ex" and released on the same night, about 1:30h after the beginning of collection. Groups of 60 adult pairs were released along 10 sites in the field border, 0.5 m away from the first soybean line and 10 m from one to another site. One week later adults and eggs were manually separated by using a small spoon and counted in soil samples (25cm x 50cm x 30cm). Ten samples were taken from the adult alignment site, and five more were taken along a line at every 5 m into the soybean field up to 25 m. In November 1995, the density of adults that had landed on the two rows of trees was evaluated over three nights. Approximately 20 adult couples were marked each night, without removing them from the plant, using "error/ex" to paint different parts of the elytron to distinguish between sample days. Plants on which mating adults were collected were also marked with different colour tags for each night. Three nights after the last marking night, adults and eggs from soil samples taken close to the marked plants and along a transect were counted at every 5 m into the soybean field up to 25 m from the edge.

Mating Sites and Distribution of the Larval Population in the Field. During the flight and mating season, in November 1995, four sites of windbreak trees where adult aggregation had been observed were selected. In order to verify if the mating place and oviposition site were associated, in early February 1996, soon after the establishment of the larvae, soil samples were taken at four sites close to the tree rows and at every 5 m along transects up to a distance of 50 m from each marked site towards the inner soybean field, for larvae counting. For each transect larvae were counted in six soil samples, similarly to what was used in the previously described experiments and collected at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 m. In those areas where trees were inside and not on the border of the field, samples were taken at the side where a larger concentration of adults in the reproductive season was observed.

Data were submitted to ANOVA within each site, and when the F-test was significant, an additional Dunnett test was used to compare the number of larvae at progressive distances from the mating sites. A Linear Regression Analysis between the number of larvae and the distance from adult aggregation sites for each area was performed. Regression equations from the different areas were compared (two-by-two) by the Student's test, which compares the homogeneity of two regression coefficients (Steel & Torrie 1981), to verify if the tendency observed could be generalized.

Results and Discussion

Influence of Plant Height on Mating Site Choice. Adult females of *P. cuyabana* chose the higher part of the vegetation for landing, attracting males and mating. Proportionally, more adults were found landing on the upper sections than on the lower sections of the vegetation. Results for the first year, when only females were counted [Fig. 1A ($F = 8.59$; $P = 0.026$)] and for the second year, when both sexes were considered [Fig. 2A ($F = 8.457$; $P = 0.008$)] were similar. During November 1994, the proportion of females observed was larger than males (2.76 females: 1 male) but the number of mated and non-mated females was not significantly different ($F = 1.907$; $P = 0.189$). The proportion of mated females, however, was larger ($F = 9.00$; $P = 0.024$) for those in the upper sections of the vegetation (Fig. 1B), suggesting that females landing on upper sections of the substrate had more chances to mate. It is important to notice that at this time the sex ratio was about

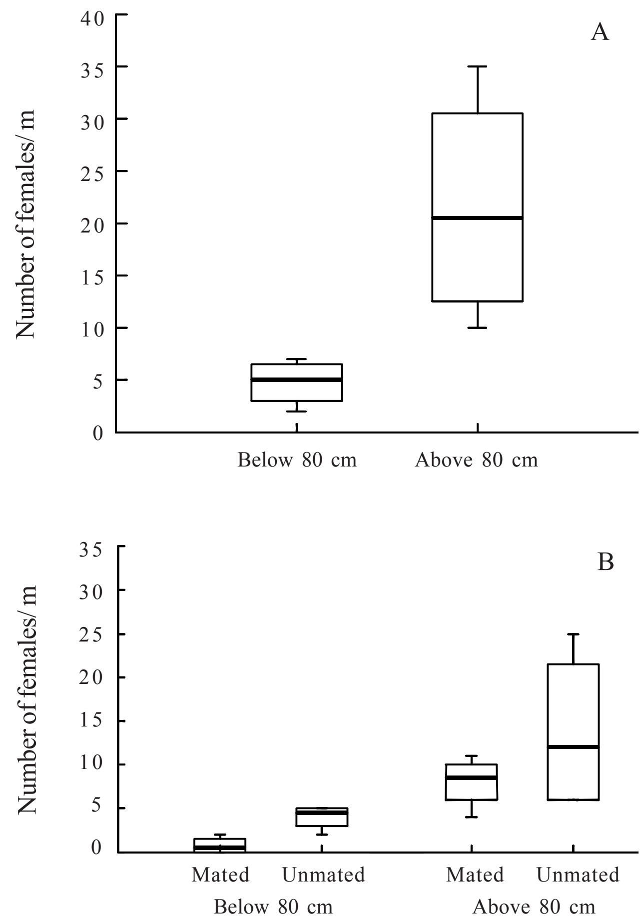

Figure 1. Position of *P. cuyabana* females on vegetation (below 0.80 m and above 0.80 m) during adult activity out of the soil. Data are for four areas in Juranda, PR. 23/nov/1994. (A - total; B - females separated as mated and unmated. Media data of 4 areas).

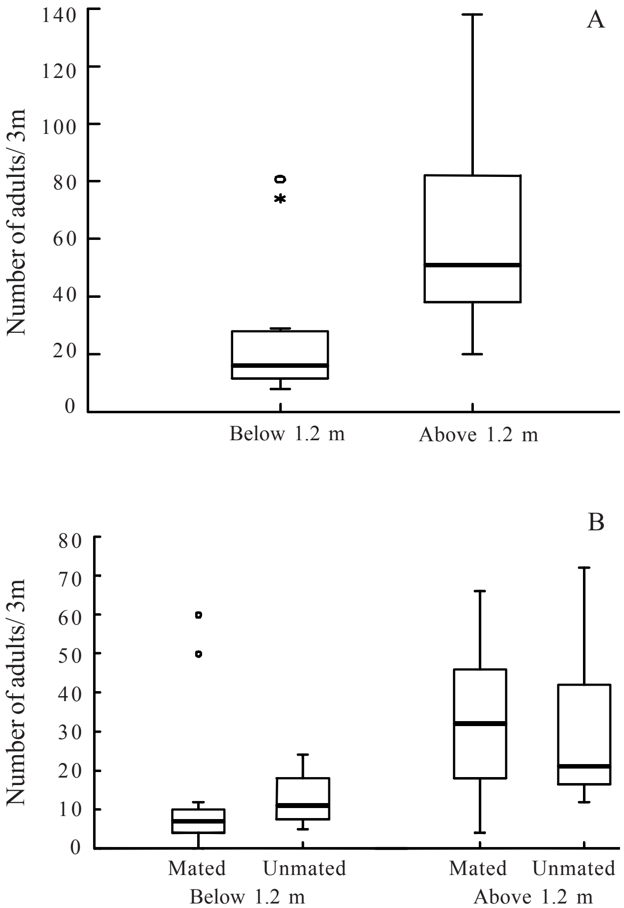

Figure 2. Position of *P. cuyabana* adults on vegetation (below 1.20 m and above 1.20 m) during adult activity out of soil, in Juranda, PR. 3/nov/1995. A - total; B - adults separated as mated and unmated. Media data of 4 areas)

three females to one male.

In 1995, when observations were carried out in the beginning of the reproductive season, the number of males was larger than females (1female: 1.76 males). Nevertheless, the total number of mating and non-mating adults was similar in both the upper and lower parts of the vegetation ($F = 0.182$; $P = 0.672$). There was a trend, (84% of cases) for the proportion of couples in the upper sections of the vegetation to be higher than in the lower sections ($F = 2.135$; $P = 0.159$) (Fig. 2B).

In both years, more adults were observed landing on one side of the upper sections of the vegetation. Moreover, the chosen side was always related to the wind direction, which would facilitate the diffusion of female pheromones. It is suggested that adults might prefer more conspicuous sites exactly because they facilitate mating.

Influence of Plant Height on the Choice of Sites for Oviposition in the Greenhouse. Although the number of adults found in the soil nearby soybean and maize plants was similar, higher plants were preferred (i.e. maize) for feeding and oviposition. Adults released in cages were similarly distributed between maize and soybean plants. In the cages, however, the proportion of eggs found close to maize plants was higher and more feeding marks were proportionally found on maize than on soybean leaves (Table 1). When soybean and maize plants used in the experiment had the same height, the number of adults ($t = 0.547$; $P = 0.59$) and eggs laid near both species ($t = 0.656$; $P = 0.52$) was similar, reinforcing the concept that the differences found among treatments could be associated to differences in plant height.

Table 1. Number (means ± SE) of *P. cuyabana* per cage and proportion of adults, eggs and feeding marks on both maize and soybean sides of the cage. (data taken ten days after releasing 15 adults pairs in each of ten cages in the greenhouse).

Parameter	Number/cage	Proportion close to the plant	
		Maize[1]	Soybean
Adults	23.0 ± 2.08	0.5 ± 0.04 A	0.5 ± 0.04 A
Eggs	15.0 ± 9.32	0.7 ± 0.09 A	0.3 ± 0.09 B
Feeding marks	172.8 ± 15.76	0.6 ± 0.03 A	0.4 ± 0.03 B

[1]15 to 20 cm higher than soybean
Means followed by the same letter on the line did not differ significantly by *t* test, at 5% probability.

In previous tests comparing soybean plants with different heights (one was 20cm taller than the other), the number of eggs nearby higher plants (10.5 ± 1.25) was also significantly higher ($t = 4.533$; $P = 0.001$) than nearby shorter plants (2.6 ± 1.25 eggs). The number of adults near shorter soybean plants was 7.83 ± 1.38 while this figure near taller soybean plants was 12.64 ± 1.79 ($t = 2.133$, $P = 0.054$). When soybean plants had the same height, the number of adults ($t = 1.082$; $P = 0.301$) and eggs ($t = 0.927$; $P = 0.372$) near plants was similar.

Relationship Between Mating Site and Distribution of the Adult and Larva Populations in the Field. The number of recaptured adults and eggs counted in the soil were small and did not provide sufficient evidence for the hypothesis that females remain close to the mating sites. Nevertheless, field observations on adult behavior after mating, strongly suggest that they dispersed over short distances in the soil. From the 1.200 adults marked in 1994, only four females and two males were recaptured, and all of them were found approximately 1m away from the releasing site. However, one of the marked insects had only fainting signs of the original mark, suggesting that the heavy rain storms that occurred during this period and the friction of the soil might have erased the ink mark on the adults. In the experiment conducted in 1995, five females and three males were recaptured from the soil. These insects were found about 1 m away from the plants they had used for mating. Eggs were detected in 38% of the samples taken close to those plants marked as mating sites (n = 50), but only in three cases marked females were found near the eggs. Three days after mating, 10 adults, representing 25% of those marked in the first night, were recaptured in the same windbreak plants where they were marked. Three of these marked adults (two females and one male) had been seen on soybean plants approximately 2 m away from plants marked as their mating site in the first night.

Oliveira & Garcia (2003) observed that after mating, males and females buried themselves in the soil until about 2 m from the copulation site (67.3 ± 9.9 cm). This indicates that after mating they had dispersed within a short distance. Larval density in the soil, on the other hand, was more clearly related with mating sites. Larval density, for all observed areas, was always higher at those nearest points of adult aggregations and were reduced with the distance from the sites where adults mated. Nevertheless, the Dunnett's test, using distance = 0 m as control, showed that significant differences between the aggregation points of adults were only found beyond 5 m distance in site A and B, 20 m for site C and 40 m for site D. The linear regression analyses showed a better trend for larvae distribution. The four sites analyzed were compared two-by-two and the Student's test showed no significant differences between the regression coefficients, indicating that the trend was similar at the different sites. This pattern was observed for three out of the four sites analyzed (Fig. 3).

These results indicate that the site of aggregation and mating, in the case of *P. cuyabana*, seems to be related mainly to the best sites for oviposition, since females tended to lay eggs close to the sites where mating occurred. This concept is reinforced by field observations carried out on females, which had performed long distance flights, when emerged in fields where the larval host plants had not yet germinated (Oliveira 1997). Patches of weeds and vegetation on the borders could also have been used as reference landmarks for adults when choosing landing sites, as occurred with *Nectopsyche albita* (Tozer *et al*. 1981).

Parker (1978) reported that females of many insect species are unlikely to be randomly distributed in their environment. Usually, they were more concentrated at sites where they were able to find resources, such as food or egg laying areas, or in places where they emerged and the behavior of males reflected this observation. However, some insects did not meet their partners at any of the seemingly logical locations (emergence, oviposition or feeding places), but instead congregated at distinctive topographical or physical features of the environment. The landmarks may have been as insignificant as a clearing in the forest or as monumental as the peak of a mountain (Thornhill & Alcock 1983). Parker (1978) argued that if females are attracted to a favored area primarily because of the advantages associated with prompt mating, then males at the periphery of the area may benefit because they tend to meet incoming females first. Male aggregations at the edge of nesting or foraging areas may have been centered on landmark points if these features were used as orientation guides for females moving through the area. Alternatively, males may initially have preferred elevated landmarks simply because they make it easier to find females. Parker (1978) suggested that encounter site conventions arise as a product of ecological factors that concentrate females spatially and determine the pattern of female receptivity. In the case of *P. cuyabana*, if higher plants have been used as landmarks, these landmarks were primarily chosen by females because they have facilitate the meeting of partners, but these sites must also be located in areas close to the larval host plant.

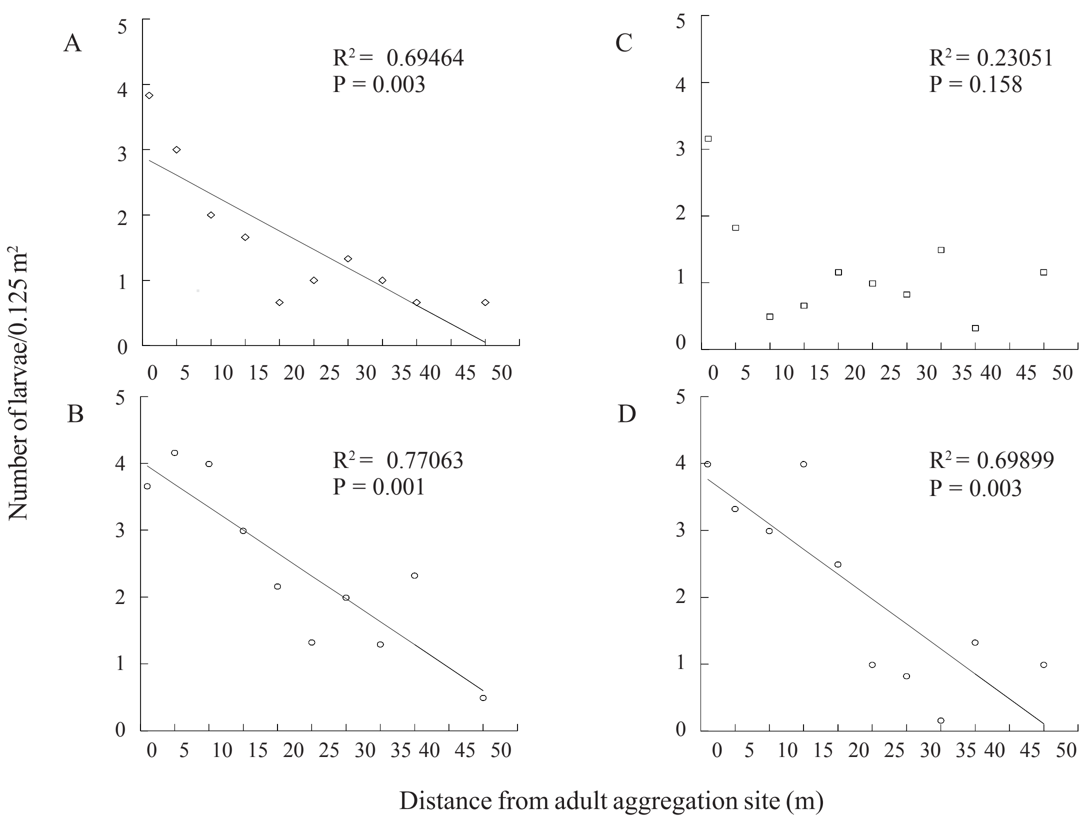

Figure 3. Relation between mating sites of *P. cuyabana* (aggregation of adults) and distribution of larvae established in the field. (Media data of 4 areas: A, B, C and D). Juranda, PR, 1995/1996.

Data from the present study suggest that female behavior is determinant for adult aggregation and for the pattern of distribution of the insect in its immature stage. For *P. cuyabana*, whose adults are nocturnal and usually disperse over small distances, it seems that the female behavior of landing on the upper parts of vegetation (taking advantage of the wind direction and vegetation close to the larval host plants), eases reproduction, probably because there is a better pheromone dispersion and a guaranteed establishment of offspring.

Adult behavior of *P. cuyabana* can be explored for subsequent management of this new native soybean pest. For instance, sowing soybean or maize along strips, 20 to 30 days prior to sowing the main crop, can provide a focus of attraction for adult aggregation. These strips could facilitate the control of the pest with the aid of biological or selective chemical control products, which could be applied solely to aggregation sites, thus reducing control costs and environmental impacts. Based on the relationships between aggregation and reproductive behavior of the adults, a trap-cropping management program could be designed for a more sustainable control of *P. cuyabana*.

Results herein presented allow the following conclusions: a) during flight, adult females of *P. cuyabana* selected plants that are more conspicuous for landing and attracting males; b) larvae and eggs density decreased as the aggregation adults site distance increased.

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