CROP PROTECTION

Feeding and Oviposition Preference of *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) on Several Crops

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Preferência Alimentar e Oviposição de *Phyllophaga cuyabana* (Moser) (Coleoptera: Melolonthidae) em Diferentes Culturas

RESUMO - Estudos em laboratório e em casa-de-vegetação foram conduzidos para estudar a preferência de *Phyllophaga cuyabana* (Moser) em se alimentar e ovipositar nas espécies de plantas: *Cajanus cajan* L. (guandu), *Crotalaria juncea* L. (crotalária), *Crotalaria spectabilis* Roth (crotalária), *Crotalaria ochroleuca* G. Don (crotalária), *Glycine max* [L.] Merrill (soja), *Gossypium hirsutum* L. (algodão), *Helianthus annuus* L. (girassol), *Stizolobium aterrimum* [*Mucuna aterrima*] Piper & Tracey (mucuna preta) e *Zea mays* L. (milho). O maior número de ovos foi observado em girassol, *C. juncea* e soja e o menor em algodão, em situação de confinamento. Em testes de multipla-escolha, os adultos não fizeram discriminação entre as plantas. Entretanto, em teste de dupla-escolha, a proporção de ovos e do consumo foliar em soja foi significativamente maior do que em *C. spectabilis*. Em situação de múltipla escolha, a distribuição das larvas ocorreu ao acaso, não sendo observada nenhuma tendência de preferência, mas em situação de dupla-escolha, usando-se soja como padrão, as larvas sempre preferiram se alimentar nas raízes de soja. Os adultos de *P. cuyabana* preferiram os hospedeiros mais adequados à sobrevivência de sua prole. Esse comportamento pode ser explorado em um programa de manejo integrado dessa praga.

PALAVRAS-CHAVE: Scarabaeoidea, praga de solo, coró, planta hospedeira

ABSTRACT - Laboratory and greenhouse experiments were carried out to study food and oviposition preference by *Phyllophaga cuyabana* (Moser) on different plant species as *Cajanus cajan* L. (pigeon pea), *Crotalaria juncea* L. (sun hemp), *Crotalaria spectabilis* Roth (showy crotalaria), *Crotalaria ochroleuca* G. Don (slenderleaf rattlebox), *Glycine max* [L.] Merrill (soybean), *Gossypium hirsutum* L. (cotton), *Helianthus annuus* L. (sunflower), *Stizolobium aterrimum* [*Mucuna aterrima*] Piper & Tracey (velvetbean) and *Zea mays* L. (mayze). In no-choice experiments, the number of eggs layed in sunflower, *C. juncea* and soybean was larger compared to cotton. Despite the fact that the adults did not discriminate among plants, in dual-choice test, the proportion of eggs layed and leaf consumption by *P. cuyabana* adults in soybean were significantly higher than in *C. spectabilis*. The larval distribution in the soil was at random in multiple-choice, withouth any trend of preference, but in dual-choice, when soybean was the control, larvae always preferred to feed on its roots. *P. cuyabana* adults had preference for more suitable hosts and that could stand their offspring survival. This behaviour can be usefully exploited in an integrated management program for this pest.

KEY WORDS: Scarabaeoidea, soil pest, white grub, host plant

Phyllophaga cuyabana (Moser) is a pest of soybean and corn in South and Central regions of Brazil. The larvae (white grubs) spend most of their life cycle below the soil surface, while the adults occur only above ground. Due to its subterranean habits, the management of the white grub is difficult. One potentially important control strategy for this pest is crop rotation, using alternative host plants (Oliveira *et al*. 2004) attractive to adults but unsuitable as food to the larvae.

P. cuyabana larvae feed on roots of various plants, like soybean, maize, sunflower, sun hemp, brazilian ragwort (*Senecio brasiliensis* Less.), radish and wheat (Oliveira *et al*. 2004). Adult females were observed feeding on leaves of soybean, maize, sunflower, sun hemp and *S. brasiliensis*

(Oliveira & Garcia 2003), but no tests regarding feeding preference of this species were performed.

Travis (1939) reported *Phyllophaga lanceolata* (Say) adults feeding on approximately thirty host plants, among them corn, soybean and potato. There are several reports that describe observations of adult *Phyllophaga* spp. feeding on the foliage of plants (Morón 1996). However, the feeding preference of *Phyllophaga* adults was not investigated.

According to Diagne (2004), the suitability of different plants for insect development and survival can vary tremendously within all possible host plants of a white grub species, but as a polyphagous insect it may show a strong preference for a relatively small number of plant species. Knowledge on the feeding preference of *Phyllophaga* adults would help in understanding the biology of the pest and perhaps explain its specific distribution among crops in different cropping systems.

The purpose of this study was to investigate food and oviposition preference of *P. cuyabana* of some plants within its host plant range.

Material and Methods

Host-plants were selected according to their occurrence in soybean production systems and to the predominant crops in the regions of occurrence of *P. cuyabana*. That includes *Cajanus cajan* L. (pigeon pea), *Crotalaria juncea* L. (sun hemp), *Crotalaria spectabilis* Roth (showy crotalaria), *Glycine max* [L.] Merrill (soybean), *Gossypium hirsutum* L. (cotton), *Helhiantus annuus* L. (sunflower), *Stizolobium aterrimum* [*Mucuna aterrima*] Piper & Tracey (velvetbean), and *Zea mays* L. (maize).

Adults and larvae of *P. cuyabana* were obtained from a laboratory colony maintained at Embrapa Soja or collected in soybean fields in Boa Esperança and Juranda, Paraná State. Laboratory assays were conducted at 25 ± 2ºC and 75 ± 2% RH. Greenhouse assays were conducted at 25 ± 2ºC and 83 ± 2% RH.

Greenhouse and field experiments were set up in completely ramdomized block design, while the laboratory assays were developed in completely ramdomized design. Treatment effects were evaluated by analysis of variance (ANOVA) using a general linear model. When ANOVA indicated a significant effect of treatments at 5% probability, the means were compared by the Tukey test.

No-choice laboratory test for oviposition. *P. cuyabana* couples were collected from soybean fields, during the first flying nights, in the pre-oviposition period. In the laboratory, they were placed in transparent acrylic pots (2 L) covered with a nylon gauze cloth. In each pot, containing soil from the area of the adult collection, one plant of each species was maintained/pot, containing two adult couples. The tested plants were: soybean, sunflower, sun hemp, showy crotalaria, cotton, and maize. The experimental design was completely randomized with nine replicates. The eggs laid in each pot were evaluated every other day, until females' death. After being counted, the eggs were transferred to petri dishes containing autoclaved sterile humid soil.

Greenhouse multiple-choice test for oviposition and adult distribution. The experiment was carried out with eight treatmens and seven replicates in the first year (multiple-choice test 1), and six treatments with ten replicates (multiple-choice test 2) in the following year. Blocks consisted of a single 150 L asbestos box, divided by celluloid strips into seven (test 1) or six (test 2) sections, placed approximately 5 cm above the soil surface and covered by a 1m high nylon gauze. One plant species was sown in each sector of the asbestos box and the species tested in both tests were soybean, sunflower, maize, cotton, and sun hemp. Showy crotalaria was evaluated only in test 2; velvetbean and pigeon pea were tested only in test 1.

Artificial infestation of adults was carried out at approximately one week after plant emergence. The number of leaf ridges (feeding signs with 0.25 cm^2 of the leaf eaten) was also evaluated in test 1. Fifty (test 1) or thirty (test 2) *P. cuyabana* adult pairs were placed in the centre of each cage whose pot was filled with humid soil. The number of eggs and adults in each sector was evaluated 14 days later. In the second year, simultaneously, a test using a similar methodology, but with 10 cages, mixing soybean and *C. juncea* or growing only soybean, was set up to evaluate the effect of the number of host species suitable for *P. cuyabana* oviposition.

Dual-choice test for oviposition and adult distribution in outdoor cages. This assay was performed using *P. cuyabana* adults collected in a soybean field during the first adult flight. Each treatment consisted of four nylon net cages (2 m width x 2 m long x 1 m height) containing six trays (30 cm width x 50 cm long x 15 cm height) of soybean and six trays of each alternate host plants (maize, sun hemp, showy crotalaria, or sunflower). The trays were placed side-by-side, simulating double mixed-cropping. Cages containing trays with only soybean were used as a control, simulating monoculture. The floor of the cages was lined with transparent plastic. Thirty *P. cuyabana* adult couples were released in the middle of each cage. The thirty cages were distributed 120 cm apart in a completely randomised design.

Over a period of ten days, each night, those adults that flew and landed on the different substrates were recorded and marked on different part of the elytra with a spot of white non-toxic correcting fluid. In the following morning, adults that were unable to burrow into the soil were also recorded and the live ones were marked on the elytra before being reintroduced into the middle of the cage. Each morning, pots were taken out from the cages and the live adults recorded; dead ones were recorded and discarded. At sunset, these pots were reintroduced into the cages with the remaining adults.

A sample of insects (60 adults), taken from the same population used in the experiment, was maintained in cages in the laboratory for estimating the preoviposition period. The first egg was observed at three days after the artificial infestation. Seven days following the first observed oviposition, the trays were removed from the cages for recording the number of live and dead adults as well as eggs, including those from the central pots. The number of leaf ridges (0.25 cm^2 of the leaf eaten) was also evaluated. The frequency of adults, eggs and feeding signs (leaf ridges) was evaluated by dividing the number observed in trays containing soybean plants by the total numbers observed in all trays. The expected frequency was 0.50, assuming that the distribution occurred independently of treatment (i.e. by chance).

Multiple-choice test for larval food preference in the greenhouse. The experiment was conducted with eight treatments and ten replicates in a randomized block design. Each block consisted of an asbestos box (150 L) divided by celluloid strips into eight sections. The treatments were soybean, maize, sunflower, cotton, pigeon pea, velvetbean and *C. spectabilis*. A single plant species was sown in each sector. In the centre of each box, joining each sector, a hole (10 cm diameter) was done, where 15 second- and third-instar larvae were placed. Two artificial infestations, consisting of seven larvae, were carried out on consecutive days. During the infestation, a portion of soil covered each larva to prevent larval wounds/lacerations due to their aggressive nature. Ten days after infestation, the larval distribution on each plant species was evaluated by recording the number of larvae in each box sector.

Dual-choice test for larval food preference in the laboratory. This experiment was conducted with 20 replicates and five treatments [roots of soybean, maize, *Crotalaria ochroleuca* G. Don (slenderleaf rattlebox), *C. juncea* and *C. spectabilis* grown in the greenhouse]. Each plot consisted of an acrylic container (15 cm x 15 cm width x 3 cm height), lined with filter paper, in which a third instar larva was placed and maintained in a growth chamber, at 25 ± 2º C, without light. A portion of soybean root and other plant roots were placed into each box. The control plot consisted of two portions of soybean roots. The roots, washed and dried with a paper towel, were replaced daily. An additional control with roots of each species, but without larvae, was used to measure any losses of root weight due water evaporation. Roots were weighted before and after the consumption period.

Multiple-choice test in the field. This experiment consisted of eight plots (18 m^2) of each treatment (soybean, maize, sunflower, cotton, sun hemp, mucuna). Each plot had six rows of 6 m of each plant species. Twice a month, from November to early April, the population of *P. cuyabana* was estimated in one sample (0,10 m^2, 30 cm depth) in each plot. One previous sample was taken in October, just before sowing, when only pupae were found in the field.

Results

No-choice laboratory test for oviposition. *P. cuyabana* female adults ate leaves from all tested plants. Females laied eggs close to any available plant species, but in the laboratory, during no-choice assays, the lowest number of eggs was observed close to cotton (Table 1). The greatest number of eggs was observed close to sunflower, sun hemp (*C. juncea*), and soybean, although soybean was only significantly different when compared to cotton.

Table 1. Distribution of eggs (mean ± SE) by *P. cuyabana* females on different plant species in a no-choice laboratory bioassay (n = 18 couples/treatment).

Plant species	Number of eggs/female
Sunflower	14.4 ± 1.05 a
Sun hemp	10.8 ± 1.52 a
Soybean	10.5 ± 1.76 ab
Maize	6.4 ± 1.33 bc
Showy crotalaria	3.4 ± 1.24 bc
Cotton	2.7 ± 0.83 c
F value	15.03 **

Means followed by the same letter on the column did not differ significantly by Tukey test, at 5% probability; ** = P < 0.01.

Greenhouse multiple choice tests for oviposition and adult distribution. When females were allowed to choose among different plants, in a multiple-choice situation in the greenhouse, *P. cuyabana* leaf consumption was significantly greater in sun hemp than in other plants (Table 2). However, the number of adults as well as the number of eggs was similar in all plant species, with no significant differences observed at minimal 5% probability in both performed tests. During the fallow period only adults and eggs were evaluated, as no leaves of the tested plants were available for insect feeding.

Dual-choice tests for oviposition and adult distribution in outdoor cages. In these tests, *P. cuyabana* consumed more leaves of soybean sowed as monoculture than in diculture of soybean and another plant species; more than twice feeding ridges were observed in soybean compared to the other tested plants (Table 3). As expected, the proportion of feeding marks in soybean monoculture was near 50%. However, when this crop was sowed in diculture with slowy crotalaria, 88.6% of feeding ridges were observed in soybean. On the other hand, only 17.5% of them were observed in soybean when pared with sunflower. In spite of the difference in the foliar consumption, the adult distribution was similar in all treatments, independently of host plant sowed paired to soybean.

The total number of eggs per cage was similar in both systems, independently of the plant species used in the system (Table 3). However, the proportion of eggs laied in relation to soybean was higher (84.1%) in the cages containing soybean plus showy crotalaria than in soybean plus sunflower (48.9%).

Dual and multiple-choice test for larval food preference in laboratory and greenhouse. The total root intake by larvae/container, was similar, regardless of the root type offered to the larvae in dual-choice test. Conversely, larvae ingested proportionally greater amount of soybean roots in the dual choice situation compared to the other roots (Table 4), and all treatments differed from soybean x soybean, whose proportion was 0.51 ± 0.039, with the expected value being 0.50.

Table 2. Number of leaf consumption marks caused by *P. cuyabana* females, number of adults and number of eggs (mean ± SE), in multiple-choice tests in caged experiments in the greenhouse (test 1, n = eight cages divided in eight sectors/treatment; test 2, n = ten cages divided in six sectors/treatment).

Plant species	Test 1			Test 2	
	Leaf	Adults	Eggs	Adults	Eggs
Sun hemp	26.9 ± 4.29 a	8.0 ± 1.51	7.2 ± 1.48	12.9 ± 1.58	7.8 ± 2.75
Sunflower	14.3 ± 3.89 b	6.5 ± 0.86	6.6 ± 2.06	8.9 ± 1.67	12.6 ± 2.78
Soybean	12.9 ± 3.21 b	9.8 ± 1.86	8.5 ± 2.49	13.6 ± 1.57	10.3 ± 1.14
Maize	10.5 ± 1.47 b	7.7 ± 1.18	8.3 ± 2.19	9.5 ± 1.87	6.6 ± 1.79
Cotton	3.8 ± 1.44 c	8.3 ± 2.01	6.6 ± 2.06	11.0 ± 2.06	9.3 ± 3.10
Pigeon pea	3.7 ± 1.19 c	8.4 ± 1.14	7.2 ± 2.24	-	-
Velvetbean	1.5 ± 0.70 c	6.0 ± 1.72	16.4 ± 4.30	-	-
Showy crotalaria	-	-	-	12.1 ± 1.16	6.0 ± 1.13
Fallow	-	5.8 ± 1.26	8.2 ± 2.96	-	-
F value	50.58 *	1.98^{ns}	1.50^{ns}	1.33^{ns}	1.30^{ns}

Means followed by the same letter on the column did not differ significantly by Tukey test, at 5% probability; * = P < 0.05; ^{ns} = not significant.

Table 3. Number of consumption marks caused by *P. cuyabana* females and number of adults and eggs (mean ± SE), in systems simulating mixed crop and single crops (n = five cages/treatment) in caged experiments (dual-choice test) outdoors.

Crop system[1]	Leaf consumption (feeding marks)		Adults	
	Total number[2]	Proportion (%) on soybean control	Total number[2]	Proportion (%) on soybean control[3]
Soybean and showy crotalaria	347.6 ± 59.38 b	88.6 ± 5.74 a	26.4 ± 4.03 b	55.7 ± 5.23
Soybean and maize	358.8 ± 58.98 b	62.0 ± 5.67 b	40.8 ± 4.45 a	52.3 ± 3.54
Soybean and sun hemp	379.2 ± 51.34 b	36.3 ± 4.03 cd	36.2 ± 1.91 ab	50.7 ± 4.45
Soybean and soybean (single crop)	802.8 ± 67.61 a	49.6 ± 4.28 bc	31.2 ± 1.96 ab	51.1 ± 1.11
Soybean and sunflower	342.6 ± 55.75 b	17.5 ± 2.78 d	36.4 ± 2.42 ab	54.9 ± 5.01
F value[4]	10,439 **	34,16 **	4.03 *	0.347^{ns}

Crop system[1]	Eggs	
	Total number[2, 3]	Proportion (%) on soybean control[3]
Soybean and showy crotalaria	48.0 ± 11.54	84.1 ± 5.21 a
Soybean and maize	50.4 ± 12.24	57.9 ±13.89 ab
Soybean and sun hemp	42.2 ± 18.87	54.2 ± 1.92 ab
Soybean and soybean (single crop)	69.6 ± 18.84	49.0 ± 4.83 b
Soybean and sunflower	33.6 ± 3.25	48.9 ± 9.47 b
F value[4]	0.848^{ns}	4.025 *

[1]Mixed crop (six soybean trays, considered as control, and six test plant trays), single crop (12 soybean trays, six considered as test plants and six considered as control); [2]considering 12 trays; [3]analysis performed with ln (x) transformed data.
Means followed by the same letter in each column did not differ significantly by Tukey test, at 5% probability; * = P < 0.05; ** = P < 0.01, ^{ns} = not significant.

Table 4. *P. cuyabana* larval consumption (mean ± SE) of roots on soybean or other plant species, in dual-choice laboratory experiments (n = 20 larvae/ treatment).

Plant species	Consumption (mg)			Proportion of soybean (control) consumption
	Total/ container	Soybean (control)	Plant test	
Soybean/maize	6012 ± 350	4351 ± 237	1751 ± 238	0.73 ± 0.03 a
Soybean/sun hemp	4829 ± 410	3569 ± 400	1260 ± 223	0.72 ± 0.05 a
Soybean/showy crotalaria	4534 ± 535	3388 ± 477	1146 ± 208	0.72 ± 0.04 a
Soybean/*C. ochroleuca*	6252 ± 310	4223 ± 359	2029 ± 257	0.67 ± 0.05 a
Soybean(control)/soybean (test)[1]	5438 ± 485	2760 ± 247	2629 ± 248	0.51 ± 0.04 b
F value	3.07 ns	-	-	5.67**

[1]Simulating single crop
Means followed by the same letter on the column did not differ significantly by Tukey test, at 5% probability; ** = P < 0.01, ns = not significant.

In multiple-choice greenhouse bioassays, larval distribution occurred mostly by chance. The number of third-instar larvae observed near roots was similar for all treatments, except that the number of larvae observed on *C. juncea* was significantly greater than on cotton (Table 5).

Multiple-choice test in the field. In November, when *P. cuyabana* adults were present, their populations were similar in all hosts in a multiple-choice situation (policulture). Later, from December to February, when only second- and third-instar larvae were observed in the soil, the population was no longer equally distributed between plots. In general, the population was higher on soybean, maize, sunflower and velvetbean plots (Table 6).

Discussion

Despite the adult distribution on the different hosts was similar in multiple-choice tests, differences in foliar consumption and oviposition preference were observed. As these activities are only exerted by *P. cuyabana* females, possibly if the population records were divided by sex, differences among treatment would be detected.

When we consider together consumption and oviposition preference of *P. cuyabana* females, sunflower seemed much more palatable and attractive compared to *C. spectabilis*, in both diculture systems. Consequently, higher number of eggs per soybean plant is expected when the crop is combined with *C. spectabilis* and a reduction of egg numbers when mixing with sunflower. Oliveira *et al.* 2004, already reported cotton and *C. spectabilis* as unsuitable hosts for *P. cuyabana* larvae.

Hierarchies of preference for plant resources of many phytophagous insects have been reported. A simple evolutionary hypothesis of variation in resource quality for offspring performance (Craig *et al.* 1989) can likely explain these preferences. Nevertheless, strong relationships between adult food preference and offspring performance have rarely been shown in polyphagous insect species (Rausher 1983, Thompson 1988, Craig *et al*. 1989). For example, the

Table 5. Number (mean ± SE) of third-instar *P. cuyabana* larvae neighbouring different plant species, in greenhouse multiple-choice experiment (greenhouse cages were divided in eight sectors).

Plant species	Number of larvae/sector
Sun hemp	3.0 ± 0.47 a
Maize	2.1 ± 0.74 ab
Pigeon pea	1.6 ± 0.23 ab
Sunflower	1.4 ± 0.43 ab
Soybean	1.3 ± 0.45 ab
Showy crotalaria	1.2 ± 0.33 ab
Velvetbean	1.1 ± 0.42 ab
Cotton	0.6 ± 0.24 b
F value	2.96 **

Means followed by the same letter on the column did not differ significantly by Tukey test, at 5% probability; ** = P < 0.01.

Table 6. Number of *P. cuyabana* / 0.5 m^2 (mean ± SE) on different plant species, in multiple-choice experiments under field conditions.

Plant species	Adults and 1st-instar larvae in November	2nd- and 3rd-instar larvae from December to early February
Soybean	1.5 ± 0.96	15.6 ± 2.16 a
Maize	2.0 ± 0.82	14.5 ± 1.70 a
Sunflower	1.5 ± 0.96	12.3 ± 1.72 a
Velvetbean	0.5 ± 0.26	8.2 ± 1.82 ab
Sun hemp	1.3 ± 0.42	5.4 ± 1.75 b
Cotton	0.6 ± 0.37	2.2 ± 0.64 b
F value	1.40 ns	10.72 **

Means followed by the same letter in each column did not differ significantly by Tukey test, at 5% probability; ** P < 0.01.

Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is attracted by a wide array of plants, regardless of their suitability as hosts to larvae. This fact suggests that acceptance or rejection of hosts occurs mainly in response to stimuli at the leaf surface (Potter & Held 2002). On the other hand, *Diloboderus abderus* Sturm (Coleoptera: Melolonthidae) female do not feed but prefers to oviposit in the areas with soybean residual biomass compared to maize. Likely, this behaviour can be explained by the greater amount of residue produced by the leguminous, which are the food supply for larvae of the benning of first instar (Silva *et al.* 1996, Silva & Salvadori 2004).

Our results showed that although *P. cuyabana*, as *P. japonica*, is a polyphagous feeder, it presented a different degree of preference for some host plant species. In general, adult females tended to consume larger amount of soybean plants and laid less eggs on soil close to less suitable hosts for larval development, such as cotton and C. *spectabilis* Roth. The larvae preferred feeding on soybean and the third-instar larvae avoided feeding on cotton. The higher populations observed on soybean, maize and sunflower plots, in field experiments, indicate that larvae survived better in these plant than in cotton or sun hemp.

Studies with *P. japonica* support the view that host range in dietary generalists is controlled mainly by the presence of deterrents in nonhost plants (Potter & Held 2002). In choice situation, *P. cuyabana* adults were capable to distinguish among species of the same genera as *C. spectabilis* and *C. juncea*, probably due to composition and concentration of some secondary chemical compounds, as pyrrolizidine alkaloid, in these plant species. *P. cuyabana* adults showed preference for more suitable hosts that could stand their offspring survival. This behaviour can be usefully exploied in an integrated management program for this pest. Those hosts, which are attractive to adults, as sunflower, can be used as a trap crop or the less suitable hosts, as cotton and *C. spectabilis*, can be cultivated in mixture or in crop rotation to reduce populations of *P. cuyabana* in soybean crops. However, before being recommended to growers, these alternatives must be further investigated in field conditions.

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