ECOLOGY, BEHAVIOR AND BIONOMICS

Alternative Food Sources and Overwintering Feeding Behavior of the Boll Weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) under the Tropical Conditions of Central Brazil

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ABSTRACT - The boll weevil causes serious damage to the cotton crop in South America. Several studies have been published on this pest, but its phenology and behavior under the tropical conditions prevailing in Brazil are not well-known. In this study the feeding behavior and main food sources of adult boll weevils throughout the year in Central Brazil was investigated. The digestive tract contents of insects captured in pheromone traps in two cotton fields and two areas of native vegetation (gallery forest and cerrado *sensu stricto*) were analyzed. The insect was captured all through the year only in the cerrado. It fed on pollen of 19 different plant families, on Pteridophyta and fungi spores and algae cysts. Simpson Index test showed that the cerrado provided greater diversity of pollen sources. In the beginning of the cotton cycle, the plant families used for pollen feeding were varied: in cotton area 1, the weevil fed on Poaceae (50%), Malvaceae and Smilacaceae (25% each); in cotton area 2 the pollen sources were Malvaceae (50%), Asteraceae (25%) and Fabaceae and Clusiaceae (25% each); in the cerrado they were Chenopodiaceae (67%) and Scheuchzeriaceae (33%). No weevils were collected in the gallery forest in this period. After cotton was harvested, the family Smilacaceae was predominant among the food plants exploited in all the study areas. These results help to explain the survivorship of adult boll weevil during cotton fallow season in Central Brazil and they are discussed in the context of behavioral adaptations to the prevailing tropical environmental conditions.

KEY WORDS: Pollen, cerrado, cotton, gallery forest, host plant

The boll weevil *Anthonomus grandis* Boheman causes serious damage to cotton yield in South America. It is a pest difficult to control due to its endophagous feeding habit. Several studies have been published on the boll weevil ecology elsewhere, but its phenology and behavior under the tropical environmental conditions of Central Brazil still has to be investigated. In the United States the boll weevil overwinters in the adult stage to survive the low winter temperatures. In the spring, as temperature rises, the insect resumes development and colonizes cotton fields (Rummel & Curry 1986). The successful eradication program of this pest in the Southeast US depends in part on the synchronization of overwintering populations for the application of control measures (Martin 1986).

A boll weevil regulatory control program is underway in the State of Goiás, mid-west region of Brazil, which also relies on the synchronization of overwintering populations that infest cotton fields in the beginning of the cotton crop (Degrande *et al* 2003). It has been suggested that, even in the warm areas of Brazil, the boll weevil also enters a state of dormancy in refuges around agricultural areas during the mild winter or dry season (Campanhola & Martin 1987, Degrande 1991, Gondim *et al* 2001). However, some adults survive and can be captured on pheromone traps after cotton is harvested (Santos 2001, Ribeiro *et al* 2006, Ribeiro 2007).

The mid-west region where most of the cotton is cultivated in Brazil is dominated by the cerrado biome, a savanna-like vegetation in which many plant species bloom during the dry season (Eiten 1994). Since boll weevil adults feed on polen, the blooming in cerrado may potentially provide an alternative source of food for this insect when cotton is not been grown. It is possible that the boll weevil has adapted or is adapting to the mild temperatures and food availability during the winter in Central Brazil by changing its overwintering behavior. In this case, the success of the currently applied control tactics will be affected.

Female ovary development depends on a pollen diet (Rummel & Curry 1986). The literature reports that this insect can only complete its life cycle when it has fed on species of the tribe *Gossypiae* (Family Malvaceae), to which cotton belongs (Luckefar *et al* 1986, Gabriel 2002). Females lay eggs inside the flower buds or bolls and the larvae feed on the developing flower or fruit tissues. The larvae feed on a small number of plant species of Malvaceae. Adults, however, feed on pollen of a great variety of plant species (Jones 1995), or puncture the cotton flower buds and bolls to feed on developing flower and fruit tissues.

To investigate food sources of the boll weevil in the United States, Hardee *et al* (1999) analyzed the pollen grain ingested by adults collected in traps throughout the year in the Mississippi Delta (USA) and found that plants of the Asteraceae (former Compositae) family were the most used food source. Additionally, pollen of many other plant families was found, such as Anacardiaceae, Chenopodiaceae, Amaranthaceae, Fagaceae, Malvaceae and Poaceae (former Gramineae). Similar results were reported by Jones & Coppedge (1996, 1998, 1999), also in the United States, and by Cuadrado & Garralla (2000) and Cuadrado (2002) in Argentina. In Brazil, where the boll weevil was introduced in the 1980s and it is still colonizing new habitats, studies developed in screen houses showed that this insect cannot complete development when fed on several Malvaceae species other than cotton (Lukefahr *et al* 1986, Gabriel 2002). However, adult males and females fed and survived on the tested species until cotton became available. Thus, plants that do not belong to the Malvaceae family can serve as food source for adult boll weevil when cotton is unavailable (Lukefahr *et al* 1986).

The objective of the present study was to follow the feeding behavioral pattern of an adult boll weevil population throughout the year in Central Brazil and to identify what kind of pollen grain was ingested by individuals collected in traps, mainly when the preferred flowering tissues of cotton plants were unavailable. This information is essential for the improvement of current methods for boll weevil population management and control.

Material and Methods

Experimental areas. This study was conducted in a 1,500 ha cotton farm (Fazenda Coperbrás, Núcleo Rural Rio Preto, Distrito Federal - 15°45'S and 47°45'W) from November 2003 to December 2004. To sample adult boll weevils throughout the year and to investigate their food sources, four study areas were chosen: two cotton areas, one native cerrado area and one native gallery forest area. Each of the cotton areas was bordered by one of the native vegetation area.

Weevils were collected using pheromone traps distributed in a grid within the study areas, starting from the Cotton Area 1 into the Cerrado Area, and from Cotton Area 2 into the Gallery Forest Area. In Cotton Area 1 and Cerrado Area, a grid of four transects with five pheromone traps along each was set, in each area. In Cotton Area 2 and Gallery Forest, the pheromone trap grid consisted of four transects with four traps each in each area, as the Gallery Forest was too narrow for five traps.

Each transect measuring 280 m started 10 m away from the border of each study area and were placed 80 m apart from each other. The pheromone traps were installed along each transect, at 70 m distance from each other, forming a grid of 20 traps in each Cotton Area 1 and Cerrado Area, and 16 traps in each Cotton Area 2 and Gallery Forest Area.

Cotton Area 1 was cultivated from November to June and Cotton Area 2 was cultivated from November to August 2004.

Boll weevil samples. Fluorescent green boll weevil Accountrap® was used with a releaser impregnated with BIO Bicudo® synthetic aggregation pheromone. The traps were installed on a pole around 1 m above the ground. A 2 cm piece of tick dog-collar impregnated with insecticide (carbamate and pyrethroids) was added to each trap to kill the captured insects. The pheromone releasers were changed every 30 days, in accordance with the manufacturer's recommendations.

Captured boll weevils were taken from the traps once a week in the months of January and February, when the cotton plants were beginning to produce flower buds, and in August and September, after the cotton fields were harvested. Since boll weevils were collected throughout the year in the Cerrado Area (Table 1), samples were also taken in May (higher flower bud production in the cotton fields) and in October and December (before cotton is sowed and after planting, but before flower bud production, respectively) for analysis of pollen content in the insect´s digestive tract.

The collected insects were taken to the laboratory where they were counted, placed into 5 ml glass vials and kept in the

Table 1 Number of adult boll weevils captured in aggregation pheromone traps from November 2003 to December 2004 in Fazenda Coperbrás – Núcleo Rural Rio Preto, Brasília, DF.

Areas	Sampling period														Total boll weevils collected
	2003		2004												
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
	Cotton intercrop				Crop						Cotton intercrop			Crop	
Cotton Area 1	-	1	4	46	70	151	243	1,018	43	224	8	1	0	0	1,809
Cotton Area 2	-	3	7	33	61	69	22	566	437	1,367	358	6	2	0	2,931
Cerrado Area	5	10	29	4	7	21	31	128	1,085	676	366	86	35	21	2,504
Gallery Forest Area	0	0	0	0	0	0	0	0	1	30	128	20	6	13	198
Total	5	14	40	83	138	241	296	1,712	1,566	2,297	860	113	43	34	7,442

freezer. To determine which plant species the adult boll weevils were feeding on when captured, a sample was randomly taken from all collected insects per month for examining the pollen grain types found in the insects' digestive tracts by using the Erdtman acetolysis process (Erdtman 1960). Sample size was determined by taking into account the total number of boll weevil captured each month in each experimental area, with a minimum of eight and a maximum of 16 insects per sample (Table 1 and Table 2). Whenever possible the pollen grains were identified to plant family, genus or species.

The acetolysis process. The acetolysis is a chemical process that dissolves the insect's body but not the pollen grains (Jones & Coppedge 1996). Boll weevils were taken from the freezer one day before being subject to acetolysis, cleansed with 99.3% ethanol and dried on absorbent paper. Each insect was transferred to a 1.5 ml centrifuge tube and crushed with a toothpick.

The following steps were conducted in a fume hood: 0.5 ml of a solution of 9:1 of acetic anhydride and sulfuric was added to each sample in the centrifuge tube, and heated at 120°C for 7 min in Termolyne®. Acetic acid was then added to stop the acetolysis reaction. Samples were centrifuged for 3 min at 4,200 *g*, and the pelleted grains of pollen were washed thrice with distilled water. After the last washing, the excess of water in each tube was discarded and two drops of Saffrin solution in ethanol was added. Samples were then centrifuged for 3 min, the supernatant discarded and eight drops of glycerin1 was added to the pellet. Samples were heated for 16h at 32°C for ethanol evaporation.

Pollen identification. Two drops of the pollen containing solution isolated were placed on a microscope slide, covered with a coverslip and fixed at the sides with nail polish. The slides were photographed using an Axophot optic microscope equipped with a camera. The identification of the pollen grains was made by using identification keys for pollen types of the cerrado flora and other publications (Heusser 1971, Salgado-Labouriau 1973, 1982, 1984, Franz *et al* 1986, Faegri & Iversen 1989, Salgado-Labouriau & Rinaldi 1990).

Table 2 Number of adult boll weevils analyzed, number of weevils with pollen in the digestive tract and number of plant families identified in each experimental area.

	Cotton Area 1	Cotton Area 2	Cerrado Area	Gallery Forest Area
Number of boll weevils analyzed	48	48	96	24
Number of boll weevils with pollen	18	21	70	13
Number of pollen grains	78	151	203	56
Number of plant families*	11	10	9	5

*Some families are common to the four areas.

Statistical analyses. Data from each month and site were statistically compared by applying the Percentage of Similarity (Renkonen Index) and the Simpson Diversity Index tests, using the Ecological Methodology software program (Krebs 1999).

Results

Alternative food sources. During cotton fallow season, the boll weevils were more frequently found in the cerrado and only for a short period of time, just after harvesting Cotton Area 2, in the gallery forest (Table 1). Of the 216 boll weevils investigated, 54% (117 insects) had pollen grains in their digestive tract. Of the 465 different pollen grains found, 80% (371) were identified to plant families. The remaining 20% (94) of the pollen grains could not be identified with the available identification keys. The different pollen grains were identified as belonging to 19 plant families. Of these, only eight genera and two species were identified to their lower taxonomic levels (Table 3). Besides pollen, seven Pteridophyta spores, 481 fungal spores and 356 algal cysts were also found in the boll weevils digestive tract.

Pollen grains from Smilacaceae were the most commonly found (66%), followed by Proteaceae (11%), Melastomataceae-Conbretaceae (7%) and Myrtaceae (6%). Most of these pollen were consumed when cotton pollen was not available. The families Fabaceae (Leguminosae), Malvaceae and Poaceae (Gramineae) formed together 5% of the pollen found in the examined samples. Other families, such as Annonaceae, Acanthaceae, Asteraceae, Chenopodiaceae, Clusiaceae, Convolvulaceae, Malpighiaceae, Moraceae, Pinaceae, Rosaceae, Saxifragaceae e Scheuchzeriaceae were each represented by less than 1% of the total pollen found, all together making up to 5% of the collected pollen.

Cotton flowering season. Fig 1 shows the percentage of pollen from each plant taxonomic group recovered from the boll weevils collected from January to February, when the cotton plants were beginning to produce flower buds. About half of the boll weevils collected had fed on pollen from plants other than cotton, even though they were captured inside the cotton fields. In this period in Cotton Area 1, pollen from Smilacaceae (25%) and Poaceae (50%) was more abundant than pollen from the cotton family, Malvaceae (25%). In Cotton Area 2, pollen from cotton (Malvaceae) was more frequently found, but in 50% of the samples Asteraceae, Fabaceae and Clusiaceae were also well represented (25%, 12.5% and 12.5%, respectively). In the same period, weevils collected in the Cerrado Area were found to be feeding on Chenopodiaceae (67%) and Scheuchzeriaceae (33%).

The comparison of the types of pollen found in weevils collected in the two Cotton Areas in Jan-Feb resulted in relatively low similarity between them (Renkonen Coefficient = 25). The comparison between these two areas and the Cerrado Area resulted in no similarity. No insects were trapped in the Gallery Forest Area in January and February, but the data showed that the boll weevils collected in the Cotton Area 2, close to the gallery forest were feeding on different families from those found in the Cotton Area 1, except for cotton pollen.

Table 3 Families, genera and species of plants identified by means of the pollen grains ingested by adult boll weevil in the four experimental areas.

Groups/family	Gender/species	Area 1	Area 2	Cerrado Area	Gallery Forest Area	Observations
Giminospermae						
Pinaceae					x	Pine trees
Angiospermae Monocotiledonae						
Poaceae nº 1				x		Grasses
Poaceae nº 2				x		"
Poaceae nº 3		x				"
Poaceae nº 4		x				"
Poaceae nº 5		x	x			"
Poaceae nº 6		x				"
Poaceae nº 7					x	"
Smilacaceae nº 1	*Smilax*	x	x	x	x	Herbaceous to "woody" vines
Smilacaceae nº 2	*Smilax*	x	x	x	x	"
Angiospermae Dicotiledonae						
Acanthaceae	*Ruellia*			x		Weedy herbs; ornamental;
Annonaceae	*Annona*	x				Fruit tree
Asteraceae nº 1			x	x		Herbaceous
Asteraceae nº 2	*Aspilia – Bacharis – Bidens*		x			Crop weeds
Asteraceae nº 3		x				Herbaceous
Asteraceae nº 4		x				"
Chenopodiaceae nº 1				x		Probably weeds
Chenopodiaceae nº 2				x		"
Convolvulaceae		x				Weed; ornamental
Clusiaceae			x			Annual or perennial herbs
Fabaceae (Leguminosae)		x	x		x	Herbs, shrubs, trees; some weeds
Malvaceae		x	x			The cotton family
Malpighiaceae		x	x			Lianas, shrubs and trees
Melastomataceae-Combretaceae		x	x	x	x	Mostly trees
Moraceae				x		Trees
Myrtaceae nº 1	*Eucalyptus*			x		Tree
Myrtaceae nº 2	*Eugenia*	x	x	x		Trees; could be fruit trees
Proteaceae nº 1		x				Shrubs and trees
Proteaceae nº 2	*Roupala montana*	x	x			Shrubs or trees
Rosaceae			x			Trees, shrubs , herbs, ornamentals
Saxifragaceae	*Chrysosplenium glecjomaefolium*	x				Herbs, ornamentals
Schechzeriaceae	*Scheuchzeria*			x		Herbaceous

During and after harvest. Fig 2 shows the identification and abundance of the pollen grains recovered from boll weevils collected in August and September, during and just after cotton was harvested. At this time of the year, a predominance of the Family Smilacaceae was observed in the Cotton Area 2, Cerrado Area and Gallery Forest Area. In the Cotton Area 1, a variety of families were found, predominantly Myrtaceae and Proteaceae.

Statistical analysis of this data resulted in high similarity between the Cotton Areas 1 and 2 (Renkonen

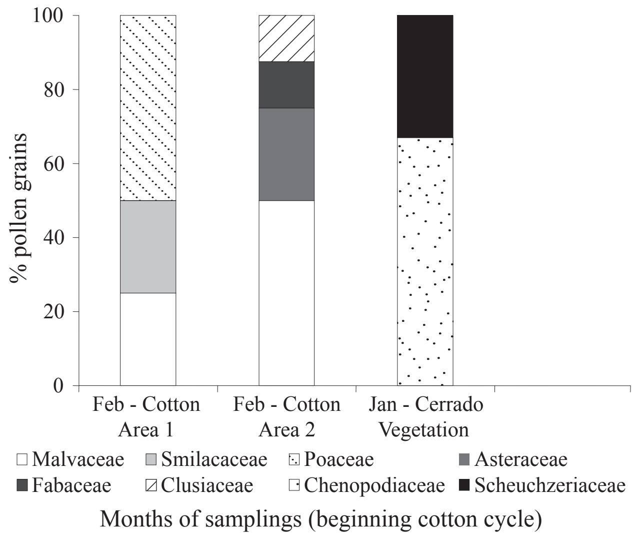

Fig 1 Percentage of pollen grains and their respective plant families found in the digestive tracts of boll weevils captured in pheromone traps in the beginning of the blooming season (January and February), in three study areas: Cotton Area 1, Cotton Area 2 and Cerrado.

Coefficient = 83.52), and low similarity between the Cotton Area 1 and the nearby Cerrado Area (Renkonen Coefficient = 11.11).

Overall, the Cerrado Area presented greater diversity of feeding plants for the boll weevil than the other study areas, both at the beginning (Simpson Index = 0.626) and end of the cotton growing season (Simpson Index = 0.743).

Other periods in the cerrado. In May, a time of high production of flower buds in the cotton fields, boll weevils were still captured in the cerrado and the most common pollen type found in their digestive tract belonged to the families Smilacaceae (95%) and Poaceae (5%). In the same area in July, just after the cotton fields were harvested, the pollen found was from Asteraceae (2%), Smilacaceae (96%) and Melastomataceae-Combretaceae (2%). In October, about the middle of the cotton fallow season, pollen of Smilacaceae (62%), Melastomataceae-Combretaceae (13%), Myrtaceae (13%) and Poaceae (12%) were found. In December, weevils collected in the cerrado had pollen of Smilacaceae (76%), Myrtaceae (12%) and Acanthaceae (12%).

Discussion

This study shows that under the environmental conditions of the cerrado biome in Central Brazil, adult boll weevils feed on pollen of 19 different angiosperm plant families throughout the year. This information on the feeding behavior of the boll weevil is relevant for the understanding of the insect's population dynamics. It indicates that the boll weevil may not always enter into a prolonged dormancy and probably leaves overwintering shelters and feed on different sources when the larval host plant is unavailable. The mild winter (average temperature of 13-23°C night/day) could not limit survivorship of adult boll weevil, although the unavailability of the larval host plant during cotton fallow season (June-July to January-February) prevents the insect from laying eggs and completing its life cycle.

Smilacaceae were the main food source for the weevils,

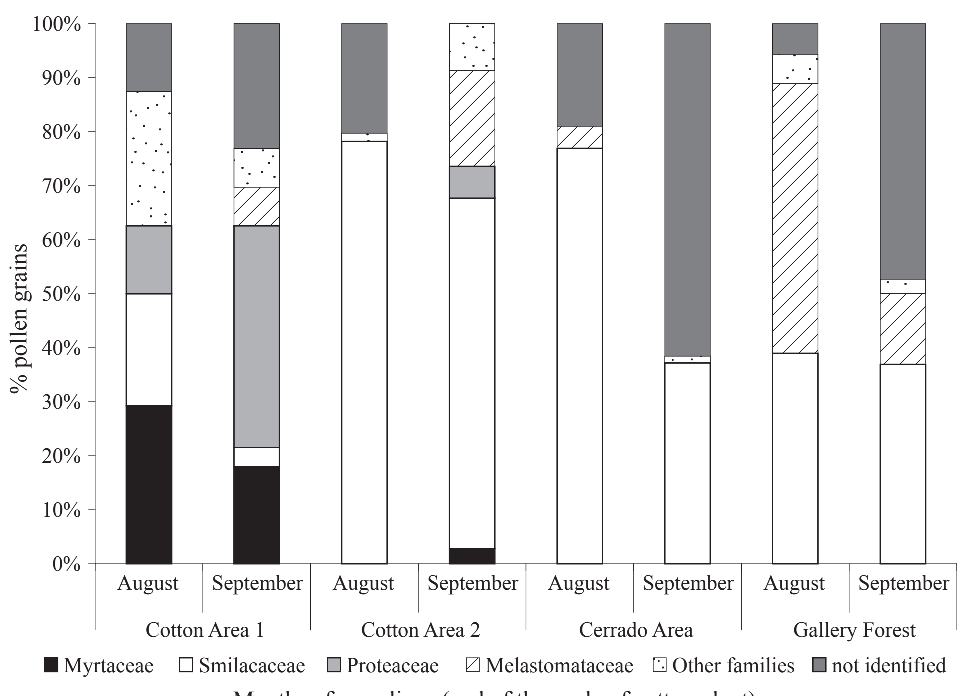

Fig 2 Percentage of pollen grains and their respective plant families found in the digestive tracts of boll weevils captured in pheromone traps after cotton harvest (August and September), in the four study areas: Cotton Area 1, Cotton Area 2, Cerrado Area, and Gallery Forest Area.

followed by Proteaceae, Melastomataceae-Combretaceae and Myrtaceae. The most frequent pollen grains found in the insects' digestive tract throughout the year belonged to *Smilax*, represented by vines, and climbers, occasionally shrubby species (Almeida *et al* 1998, Souza & Lorenzi 2005). In the cerrado and gallery forest, a few *Smilax* species bloom throughout the year, becoming a highly predictable source of food for the boll weevil. After cotton is harvested, *Roupala montana*, which blooms from March to November (Almeida *et al* 1998), and other Proteaceae, are the second most important sources of pollen, followed by *Eugenia* (Myrtaceae), which blooms between September and October (Almeida *et al* 1998).

This study also shows that adult boll weevils may feed on Pteridophyta and fungal spores and algal cysts when cotton fields are fallow. The tree trunks in the cerrado are usually covered with lichens, and that is probably the source of this kind of food.

Analysis of the average height of the plant families and species listed on Table 3, and the distribution of the pollen types found throughout the year in the boll weevil digestive tract (Figs 1 and 2) showed a tendency for the insect to feed on herbaceous plants in the period between cotton sowing and flowering (late November trough February). Except for Smilacaceae, which were commonly seen on the edges of the Cerrado Area and Gallery Forest Area, and alongside the dirty road that borders the cotton fields, many plant families, genera, and species in the agricultural settings identified as food sources for the boll weevil at this time of the year are known to be invasive (Table 3). For instance, most Asteraceae and Poaceae are weeds and invade cotton fields (Fontes *et al* 2006). In Clusiaceae, the bushy weed *Hypericum* sp. was common in the field margins. Fabaceae (formerly Leguminoseae) was also well represented by herbaceous species in the area (Fontes *et al* 2007).

Woody plant families, from which some were fruit trees and ornamentals, were more frequently exploited as the year progressed and the dry season began (Table 3). During August and September, almost all herbaceous plants around the agricultural fields were dead. On the other hand, many plant species of the cerrado biome blooms in the dry season.

Among the two cotton areas and the two areas of native vegetation surveyed in this study, the boll weevil was captured throughout the year only in the cerrado. Overall, the Cerrado Area also presented greater diversity of feeding plants for the boll weevil than the other study areas, both at the beginning and at the end of the cotton growing season, as shown by the results of the Simpson Index tests. An analysis of the plant families identified added to our own studies (Fontes *et al* 2007), and additional personal observations of the vegetation in the study areas suggested that this may be due to a preference for small-sized plants such as herbs, vines and bushes, which are scarce in the gallery forest and absent or dry in the fallow cotton fields.

There was a relatively low similarity of the types of pollen grains found in weevils' digestive tract between the two Cotton Areas and among each of the cotton Areas and the Cerrado Area. These results could be interpreted as an indication that the insects were feeding on herbaceous plants and weeds in and around the cotton fields. The composition of this flora slightly differed among the two cotton areas. Those insects that were still in the cerrado, perhaps coming out of overwintering shelters, fed on plant families typical of the cerrado biome.

During August and September, just after the cotton fields were harvested, weevils collected in the Cotton Areas 1 and 2 were found to be feeding on different plant families, while those collected in the cerrado and gallery forest were mainly feeding on Smilacaceae. We speculate that blooming plants from this family are probably the most abundant at this time of the year, although this needs to be further investigated.

A probable preference for lower height food sources could be one of the reasons for the low number of insects collected in the gallery forest, as these forests have few herbaceous plants and grasses. However, other factors such as the type of trap used and its position at one meter above the ground, away from the flowers of high trees, could have contributed to this result.

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