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The behavior of *Aphis gossypii* and *Aphis craccivora* (Hemiptera: Aphididae) and of their predator *Cycloneda sanguinea* (Coleoptera: Coccinellidae) in cotton-cowpea intercropping systems

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ABSTRACT

The intercropping is an important cultural practice commonly used in pest management. It is based on the principle that increased plant diversity in the agro-ecosystem can lead to reductions of pest populations in the crop. The current study aimed to assess the impact the colored fiber cotton-cowpea intercropped systems on *Aphis gossypii* and *Aphis craccivora* and on their predator *Cycloneda sanguinea* and the losses and the dispersion behavior of these aphids and their predator in these cropping systems. The experiment had a randomized block experimental design with two bioassays and four treatments. The number of apterous and alate aphids (*A. gossypii*) per cotton plant was 1.46 and 1.73 or 1.97 and 2.19 times highest in the solid cotton system than that found in the cotton-cowpea intercropped systems (S1) and (S2), respectively. On the other hand, the cotton-cowpea intercropped systems (S1 and S2) reduced, respectively, in 43% and 31% the number of apterous *A. gossypii* per cotton plant compared to the control. Implementing cotton-cowpea intercropped system in the S1 scheme reduced *A. gossypii* infestation, favored the multiplication of *C. sanguinea*, and allowed obtaining heavier open bolls.

Key words: aphids, cowpea, cotton, behavior, intercropping.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) cultivars with naturally colored fibers now have worldwide demand in the textile industry and have added value to the crop for small farmers (Fernandes et al. 2012a, Ramalho et al. 2012a). Cowpea (*Vigna unguiculata* L. Walp.) fixs nitrogen to the soil and it is a protein supply for people in northeastern Brazil (Braga et al. 2007, Frota et al. 2008). These agricultural products contribute to employment and income of farmers in this Brazilian region. However, *Aphis gossypii* Glov. (Fernandes et al. 2012a) and *Aphis craccivora* K. (Hemiptera: Aphididae), respectively, have been reducing cotton cowpea production in Brazil. These aphids cause direct damage by sucking sap and indirect ones by virus transmittion and excreting honeydew

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that favors the proliferation of leaf stomata-obstructing fungi (*Capnodium* spp) (Kitajima et al. 2008, Fernandes et al. 2012a, 2013, Bachmann et al. 2014, Malaquias et al. 2014).

Aphis gossypii damages cotton plants during both vegetative and reproductive stages (Ramalho et al. 2012a, Fernandes et al. 2012b) and many plant species including eggplants and okra (Leite et al. 2006, 2007). Infestation in vegetative stages turn leaves shriveled, whereas infestation in reproductive stages, especially at fruit opening, fouls and reduces fiber quality (Almeida 2001). Severe attacks by *A. gossypii* may also reduce leaf area and biomass, besides branching and plant height, harming the crop and results in economic losses (Sarwar et al. 2014).

Aphis craccivora initially infests cowpea seedlings, but as this plant develops, it may infest flowers and pods (Berberet et al. 2009). Its feeding causes leaf shriveling and bud deformation and as the population increases, plant infested become weaked due to loss of sap and toxins injected (Silva et al. 2005). *Aphis craccivora* is an efficient virus vector, transmitting *Cowpea aphidborne mosaic virus* (CABMV) (Kitajima et al. 2008), and *Blackeye mosaic virus* (BICpMV) (Lima et al. 1981). *Aphis craccivora* can decrease seed quality and plant productivity in commercial bean plants (Obopile 2006, Laamari et al. 2008) with yield losses as high as 50% (Berlandier and Sweetingham 2003, Obopile 2006).

In Northeastern Brazil, aphid are usually controlled with non-selective synthetic insecticides that are effective against pests but often cause mortality in beneficial insect (Ullah and Paul 1985, Leite et al. 2010). Agro-ecosystem diversification through intercropping may reduce insect pest infestations without the negative impacts of chemical measures (Gonzaga et al. 1991, Medeiros et al. 2009, Fernandes et al. 2012c, Ramalho et al. 2012a, b). The possibility of intercropping colored fiber cotton with fennel (*Foeniculum vulgare* Mill.) was studied (Ramalho et al. 2015) but the use of cowpea for the management of *A. gossypii* and *A. craccivora* populations has not been explored. Naturally colored cotton is ecologically friendly because it eliminates the dyeing process in industrial production with frequently (and perhaps incorrectly) chemicals use dangerous to humans (Horstmann 1995).

Well-structured and connected habitat may decreases interspecific competition by allowing species to segregate spatially (Boeye et al. 2014). In contrast, different plant species in intercropped may change arthropod communities favoring the natural enemies (predators and parasitoids) and reducing pest populations (Gonzaga et al. 1991). Intercropping can also increase the productivity of agricultural crops (Ramalho et al. 2012a, b).

Intercropped plants may repel pests or attract natural enemies (Kadam et al. 2014). Predatory lady beetles (Coleoptera: Coccinellidae) may move between crops (Bastola et al. 2014). Coccinellid may be attracted to particular crops due to prey availability and plant features such as the resource provision; shelter, protection and feeding sites (Resende et al. 2012), and plant odors can attract predators in intercropped systems (Ninkovic and Pettersson 2003). The impact of intercropping colored fiber cotton with cowpea on *A. gossypii* and *A. craccivora* populations and on their predator *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) and the dispersal of these insects among cropping systems were evaluated. The hypothesis were that: a) cotton intercropped with cowpea reduces *A. gossypii* populations in cotton, and that b) cotton intercropped with cowpea increases *C. sanguinea* abundance in the cotton crop.

MATERIALS AND METHODS

STUDY LOCATION AND COTTON AND COWPEA CULTIVARS

The study was conducted in a greenhouse at the Entomology Department of Luiz de Queiroz

Agriculture College (ESALQ - Escola Superior de Agricultura Luiz de Queiroz/USP), Piracicaba, São Paulo, Brazil. Cotton plants with naturally colored fibers (cv. BRS Safira) and cowpea (cv. BRS Itaim : (upright)) were cultivated in plastic bags with dimensions 40 x 40 x 30 cm, irrigated daily and placed in a greenhouse at 30 °C, 70 ± 10% R.H, and 12 h photophase.

APHIDS AND PREDATORS

Apterous *A. gossypii* and *A. craccivora* and one predator species (*C. sanguinea*) were used. The *A. gossypii* and *C. sanguinea* specimens were collected in a cotton field near the Entomology Department at ESALQ - USP, Piracicaba, São Paulo State, Brazil and *A. craccivora* specimens on cowpea plants in an experimental area of Embrapa Algodão (Embrapa Cotton), Lagoa Seca, Paraíba State, Brazil. Both aphid species were grown on their host plants, i.e. cotton and cowpea, respectively in cages coated with anti-aphid plastic screening at 30 ± 1 °C, 70 ± 10% R.H. and 12 h photophase. The aphids were monitored daily on the plants, and individuals were separated whenever it was necessary by taking into account their life cycle stage (nymphs and adults). The predator was reared on its preys (*A. gossypii* or *A. craccivora*) under similar conditions to those used to rear aphids.

BIOASSAYS

Effects of cotton, cowpea and cotton intercropped with cowpea systems on aphid (A. gossypii *and* A. craccivora) *populations*

The current study used a randomized block experimental design with four treatments. The bioassay 1 had the following cropping systems: S1- two cotton plants: two cowpea plants within the row- each row began and ended with two cotton plants; S2- two cowpea plants: two cotton plants within the row, alternate rows starting and ending with two cowpea plants or two cotton plants; S3- a cotton row: one cowpea row; and S4- cotton (control) (Fig. 1). Bioassay 2, had the same treatments of bioassay 1, except for S4, which was named S5- cowpea (Fig. 1).

The cotton-cowpea intercropped experimental units had rows with two cotton plants alternating with two cowpea plants within each row or of a cotton row alternating with a cowpea one (Fig. 1). Cotton and cowpea rows were spaced 40 cm from each other in both non-intercropped and intercropped plots. The spacing between plants within each row was 20 cm. The distance between the experimental units was 100 cm (Fig. 1). The experimental units were kept in a transparent plastic cage protected with white voal.

The cotton and cowpea seeds were planted in plastic pots (40 x 40 x 30 cm) and the plants were irrigated every other day.

Thirty-five days after plant emergence or at the end of the vegetative stage, 15 apterous aphids were released in a central plant randomly taken from cotton (S4) or cowpea (S5) plots (Fig. 1) in the non-intercropping systems. Thirty apterous aphids were released in the intercropping system plots (S1, S2 and S3) (Fig. 1), 15 *A. gossypii* and 15 *A. craccivora,* respectively.

LADYBUGS DISPERSION AND PROGENIES IN COTTON, COWPEA AND COTTON-COWPEA INTERCROPPED SYSTEMS

Three days after releasing the aphids (bioassays 1 and 2), 38 ladybug couples were marked (with corrective ink of different colors) and released in the crops. They were distributed in equal numbers in 15 distinct points, one in each plot. The plant with ladybug release differed from those with the aphids release within each plot.

Five colors were used to mark the forewing of each predator according to the treatment, i.e., a randomized block experimental design with five treatments (crop systems) (colors): black (S1),

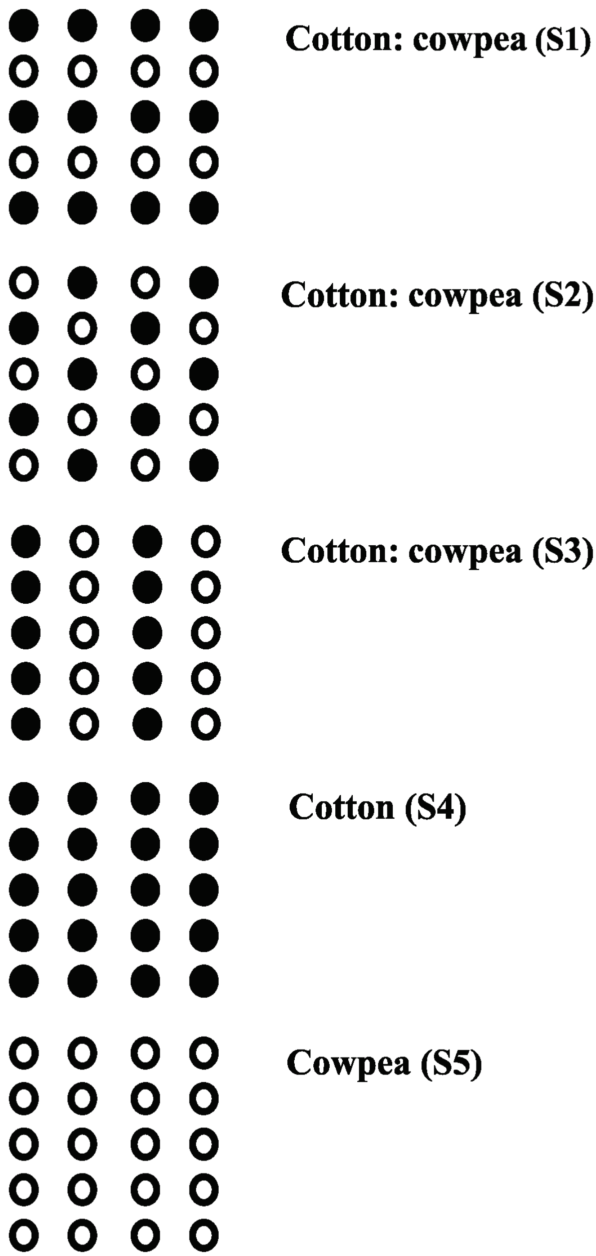

Figure 1 - Layout of experimental units in the cotton–cowpea intercropping systems and monoculture. Cotton with colored fibers (closed circles) and cowpea (open circles).

white (S2), red (S3), green (S4) and blue (S5). The distinction between blocks was done according to the scores each insect received. In the S1 cropping system, for instance, insects marked with black color scored 1, 2 and 3 and they were released in blocks I, II and III, respectively. Similar procedure was used for the other colors.

The dispersion of predators in the systems with only colored cotton (S4) and cowpea (S5) and in colored cotton-cowpea intercropped systems (S1, S2 and S3) was evaluated at three, 12 and 24 h after its release. All individuals captured were quantified and again released in three and 12 h evaluation intervals. Twenty-four hours after they were released, all the ladybugs were recaptured and discarded, except for the eggs deposited by couples during the movement bioassay. The aphids and the predator descendants remained on the plants. Other individuals of the predator were released after the first flowers emerged in the cowpea plant and after the first squares emerged in the cotton plant (approximately 65 days after the plant emergence). Adult predators marking, evaluation and disposal procedures were the same as those used in the vegetative stage.

Forty-nine days after the aphids were released (*A. gossypii* and *A. craccivora*) and 42 days after the adult predators were released and recaptured, the same-stage descendants were respectively quantified on 25 and 18 plants per plot.

DATA ANALYSIS

The *C. sanguinea* records in all cropping systems were analyzed using PROC FREQ (Sas Institute 2006). The proportions of insects found in the different cropping systems were subjected to the Pearson χ^2 test (Sas Institute 2006) to investigate the cropping system dependence for the predator dispersal in non-intercropped or in intercropped crops. The aphid and predator progenies and the cotton and cowpea plants' reproductive parameters showed normality (Shapiro-Wilk test) and homogeneity (Hartley test). The data were subjected to variance analysis (ANOVA) and the means compared using the Student-Newman-Keuls test (SNK, $P = 0.05$).

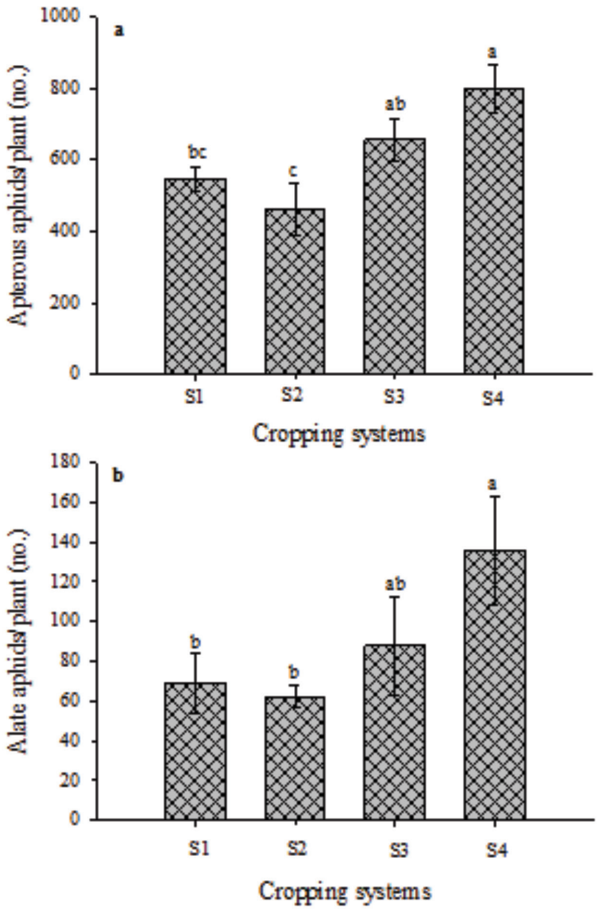

Figure 2 - Mean numbers (±SE) of apterous (a) and alate (b) *Aphis gossypii* (Hemiptera: Aphididae) in the cropping systems of cotton (S4) and cotton intercropped with cowpea systems (S1, S2, and S3). Each column represents the mean number of insects found in 25 plants. Error bars indicate SE. Within each figure, different letters indicate differences (SNK test; $P = 0.05$).

RESULTS

The number of apterous ($F_{(3, 2)} = 11.01$; $P = 0.0075$) and alate ($F_{(3, 2)} = 5.49$; $P = 0.0372$) *A. gossypii* found per plant differed among cropping systems (Table I). The highest number of aphids per cotton plant was recorded in the solid cropping system (S4) (Fig. 1) (799 apterous and 136 alates) than in the intercropped systems (S1) (Fig. 1) (545 apterous and 69 alates) (Fig. 2) and (S2) (Fig. 1) (461 apterous and 62 alates) (Fig. 2). However, the number of aphids per cotton plant quantified in the cotton-cowpea intercropped cropping system (S3) (Fig. 1) (656 apterous and 88 alates) (Fig. 2) was similar to that of the solid cotton system (S4) (Fig. 1) (798 apterous and 136 alates) (Fig. 2). The number of apterous and alate aphids (*A. gossypii*) per cotton plant was 1.46 and 1.73 or 1.97 and 2.19 times higher in the solid cotton system (S4) (Fig. 1) than in the cotton-cowpea intercropped systems (S1) and (S2) (Fig. 1). On the other hand, the cotton-cowpea intercropped systems (S1 and S2) reduced, respectively, in 43% and 31% the number of apterous *A. gossypii* per cotton plant (Fig. 3a). However, the reduction in the number of alate *A. gossypii* per cotton plant by the cotton-cowpea intercropped systems (S1 and S2) were, respectively, 53% and 47% (Fig. 3b). This indicates that the cotton-cowpea intercropped systems (S1 and S2) (Fig. 1) may be used to reduce *A. gossypii* populations.

The cropping systems did not affect the mean number of cotton bolls per plant ($F_{(3,2)} = 4.46$; $P = 0.0569$). However, they affected the mean weight of open bolls per plant ($F_{(3,2)} = 16$; $P = 0.0029$). The S1 was the cropping system with the highest open boll weight (4.59 g) which was lowest in the S2 (2.49 g), S3 (3.19 g) and S4 (2.93 g) (Fig. 4). This indicates that cotton-cowpea intercropped system may be a good tactic to produce heavier open bolls and, therefore, it may help increase cotton yield.

The mean number of apterous ($F_{(3,2)} = 3.22$; $P = 0.1040$) and alate ($F_{(3, 2)} = 2.05$; $P = 0.2088$) *A. craccivora* per plant (Table I) and weight of seed/plant ($F_{(3,2)} = 0.35$, $P = 0.7897$) showed that the cowpea did no affect these parameters. It indicates that the colored fiber cotton-cowpea intercropping systems did not affect the mean number of *A. craccivora* and the seed production per cowpea plant.

The predator movement in the vegetative and reproductive stages in all cropping systems showed that its dispersion occurred independently in the cotton-cowpea intercropped systems and in the solid cowpea system (Table II). On the other hand,

TABLE I
Summarized model of the one-way analyses of variance (ANOVA) for the effects of crop systems (cotton or cowpea and cotton intercropped with cowpea) on the populations of apterous and alate *Aphis gossypii* and *Aphis craccivora* (Hemiptera: Aphididae).

Sources	Models	df	F ratio	Prob > F
Number of apterous *A. gossypii* per cotton plant	Model	5	7.29	0.0157
	Crop system	3	11.01	0.0075
Number of alate *A. gossypii* per cotton plant	Model	5	4.17	0.0546
	Crop system	3	5.49	0.0372
Number of apterous *A. craccivora* per cawpea plant	Model	5	2.43	0.1554
	Crop system	3	3.22	0.1040
Number of alate *A. craccivora* per cawpea plant	Model	5	1.31	0.3722
	Crop system	3	2.05	0.2088

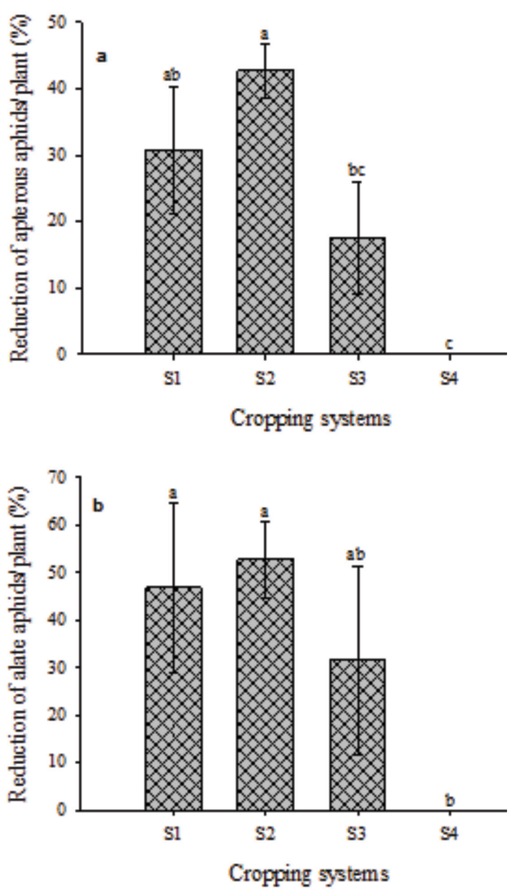

Figure 3 - Mean reduction (±SE) (%) of apterous (a) and alate (b) *Aphis gossypii* (Hemiptera: Aphididae) in the cropping systems of cotton (S4) and cotton intercropped with cowpea systems (S1, S2, and S3). Error bars indicate SE. Within each figure, different letters indicate differences (SNK test; $P = 0.05$).

TABLE II
Dispersion of *Cycloneda sanguinea* (Coleoptera: Coccinellidae) in the crop systems of cotton, cowpea and cotton intercropped with cowpea.

Stage	Predator dispersion[1]	N	DF	χ^2	P
Vegetative	Crop system (S1)	56	2	0.76	0.6832
	Crop system (S2)	45	2	2.43	0.2965
	Crop system (S3)	49	2	0.50	0.7770
	Crop system (S4)	32	2	6.33	0.0421
	Crop system (S5)	41	2	3.18	0.2036
Reproductive	Crop system (S1)	39	2	0.37	0.8320
	Crop system (S2)	39	2	1.47	0.4792
	Crop system (S3)	39	2	2.30	0.3168
	Crop system (S4)	35	2	1.95	0.3774
	Crop system (S5)	36	2	0.30	0.8623

[1]Crop systems (S1, S2 and S3) = cotton-cowpea intercropping systems, crop system (S4) = cotton, crop system (S5) = cowpea. $P < 0.05$ = the predator dispersion was dependent; $P > 0.05$ = the predator dispersion was independent.

the predator dispersion in the solid cotton system differed among crop phonological stages (Table II). The predator dispersion in the solid cotton system depended on the crop vegetative stage ($\chi^2_{(df=2)} = 6.33$; $P = 0.0421$); whereas its dispersion in the solid cotton system reproductive stage was independent ($\chi^2_{(df=2)} = 0.30$; $P = 0.8623$). Therefore, the predator dispersion in the cotton crop may vary depending on the cropping system and on its phenological stage.

The number of offspring from predators released differed between cropping systems ($F_{(4, 2)} = 4.11$; $P = 0.0317$). The mean number of *C. sanguinea* recovered per plant was higher in the S1 cropping system (2.48 individuals) than in the S2 (0.75 individuals), S3 (1.17 individuals), S4 (0.61 individuals), and S5 (0.56 individuals) cropping systems (Fig. 4). This indicates the S1 cropping system as the most suitable for this predator, probably due to better conditions and attraction to host plants.

DISCUSSION

The intercropping of different plant species is an important cultural practice commonly used in pest management; it is based on the principle that the reduction of insect pest populations in the crop may occur due to increased agro-ecosystem diversity (Risch 2005). Diversification practices, such as cotton intercropped with other crops, reduce damages by the insect pests (Ramalho et al. 2012a, b).

The implementing of cotton-cowpea intercropped system in the S1 scheme (Fig. 1) reduced *A. gossypii* infestation (Fig. 2, Fig. 3a, Fig. 3b) and favored multiplication of the *C. sanguinea* predator (Fig. 5), and consequently allowed obtaining the highest open boll weight (Fig. 4). The microclimate and plant dispositions in the plots helped to increase natural enemies population by better food and shelter and more adequate microclimate (Cividanes and Yamamoto 2002, Barbosa et al. 2011, Manjula and Lakshmi 2014). Similar results were reported by Ramalho et al. (2012a, b), Fernandes et al. (2013), and Mitiku et al. (2014), which reported the importance of using cotton cropping systems intercropped with other plant species to reduce the number of aphids in agricultural ecosystems. Intercrops like cotton with colored fibers and cowpea recorded higher populations of the predator *C. sanguinea.* This is an associational resistence, refering to the reduced herbivore attack experienced by a plant when they associated with taxonomically different species (Kaitaniemi and Piihimaki 2007), and it may be true for the intercropping systems. Associational resistance is due to resource concentration, the natural enemy hypothesis, or both (Ramalho et al. 2012a, b).

Choosing the intercropping system is very important to increase the density of natural enemies in the agro-ecosystem and to reduce the damages by aphids (Ramalho et al. 2012b). Cotton seed production was satisfactory when cotton was intercropped with sesame (*Sesamum orientale* L.) in the cultivation ratios of 3:1 and 2:1 (1148 kg ha^{-1} and 993 kg ha^{-1}, respectively) (Aladakatti et al. 2011). However, higher cotton production per plant was obtained with cotton-cassia (*Cassia angustifolia* L.) intercropped system (1094 kg ha^{-1}) than with solid cotton system (687 kg ha^{-1}) (Rathod et al. 2011).

The predator *C. sanguinea* movements in the vegetative and reproductive stages of the plants in all cropping systems showed dispersion in the cotton-cowpea intercropped systems and in the cowpea system independently. Predator movement between two habitats might be bidirectional, despite the approach involving its dispersion in the crops (Bastola et al. 2014). However, the literature shows no reports of similar crop settings to those used in the current study. When new crop combinations are set in a particular area, they may provide

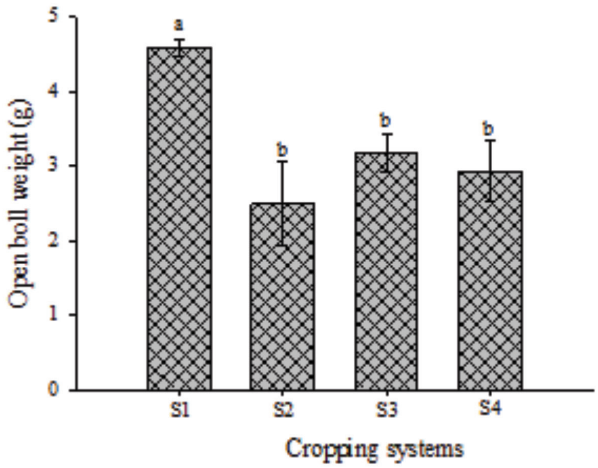

Figure 4 - Mean weight (±SE) open cotton bolls in the cropping systems of cotton (S4) or cotton intercropped with cowpea systems (S1, S2, and S3) infested by *Aphis gossypii* (Hemiptera: Aphididae). Each column represents the mean weight of cotton open bolls harvested in 12 plants. Error bars indicate SE. Different letters indicate differences (SNK test; *P* = 0.05).

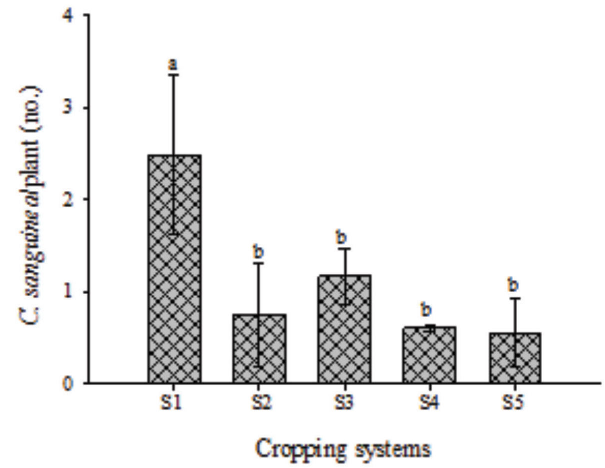

Figure 5 - Mean number (±SE) of *Cycloneda sanguinea* (Coleoptera: Coccinellidae) per cotton or cowpea plant on the cropping systems of cotton (S4) or cowpea (S5) and cotton intercropped with cowpea systems (S1, S2, and S3). Each column represents the mean number of *C. sanguinea* on 18 plants. Error bars indicate SE. Different letters indicate differences (SNK test; *P* = 0.05).

benefits to the agricultural system since the natural enemy populations found in adjacent areas can quickly colonize these new habitats (Togni 2014). Intercropping systems with pest hosts and-non host plants may enable pest movements to new habitats (Togni 2014). This increases the pest vulnerability to natural enemies and the opportunity for predators to find and predate them (Straub et al. 2014). On the other hand, predator actions may increase the pest dispersion in agricultural crops (Otsuki and Yano 2014).

The *C. sanguinea* dispersion showed that this predator has high probability to move to several intercropping habitats, i.e., cotton-cowpea intercropped systems (S1, S2, and S3) and cowpea system (S5). Cotton-cowpea intercropped systems (S1, S2, and S3) increased predator chances of finding food resources, i.e., prey besides colonization because prey will be more vulnerable in these conditions. Therefore, understanding the dispersion of predators can benefit insect conservation programs in agricultural habitats and its crucial for pest management in complex agro-ecosystems (Chailleux et al. 2014, Choate and Lundgren 2014).

Natural enemy affect aphid populations by killing or inducing their dispersion (Duarte et al. 2014). This probably occurred with *A. gossypii* in cotton-cowpea intercropped systems (S1 and S2) (Fig. 1, Fig. 3a and Fig. 3b), but not with *A. craccivora,* because this last aphid had similar densities in cowpea plants in cotton-cowpea intercropped systems (S1, S2 and S3) and in solid cowpea system (S5) (Table I).

The cropping systems tested did not affect the weight of seeds/cowpea plant ($F_{(3,2)}$ = 0.35, *P* = 0.7897). However, they benefited cotton plants, especially by attracting *C. sanguinea* (Table II and Fig. 5) as reported for this predator in *Brassica oleracea* (L.) cultivars (Azeredo et al. 2004) and the presence of this predator might be linked to specific genotypes.

Plant odor attracts natural enemies (Boullis et al. 2015). Ladybugs can learn to associate the odors of plants infested by different aphid species even under restricted environmental condictions

(Glinwood et al. 2011). This behavior by ladybugs may explain why *C. sanguinea* was attracted to the cotton-cowpea intercropped systems.

We conclude that implementing cotton-cowpea intercropped system in the S1 scheme reduces *A. gossypii* infestation, favors the multiplication of *C. sanguinea* predator, and allows the obtaining of heavier open bolls.

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