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FORESTRY SCIENCE

Dolichopodidae abundance in different cover crop species

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ABSTRACT: *This study evaluated the Dolichopodidae abundance in different cover crop species. For this, the following species were used in treatments: Crotalaria (Crotalaria spectabilis), cowpea (Vigna unguiculata), sunflower (Helianthus annuus), castor bean (Ricinus communis) and buckwheat (Fagopyrum esculentum). The survey was carried out in a field during two seasons 2012/2013 (November/December to March) and 2013/2014 (October/November to February). The Dolichopodidae abundance were evaluated weekly using a sweep net and Moericke traps. In addition, the phytophagous abundance (Thysanoptera: Thripidae; Hemiptera: Aleyrodidae and Hemiptera: Aphididae) was quantified, to allow for an inference about density-dependence. In total, 13,987 long-legged flies (96.5% in Moericke traps) were captured, represented only by the species Condylostylus erectus Becker, 1922 (Diptera: Dolichopodidae). In general, the higher abundance of C. erectus was observed in buckwheat and cowpea. Regarding the phytophagous species, 48,371 individuals [Thripidae (77.5%), Aleyrodidae (20.2%), and Aphididae (2.5%)] were quantified and a clear correlation with the C. erectus population was not evidenced. Thus, these results suggested that buckwheat and cowpea make resources available to long-legged flies, as discussed in our research. Finally, the buckwheat and cowpea sowing are options to increase the abundance of C. erectus predator populations in agroecosystems.*
Key words: *conservation biological control, environmental management, biodiversity, natural enemies, attractive crop species.*

Abundância de Dolichopodidae em diferentes espécies de adubos verdes

RESUMO: *O objetivo deste estudo foi avaliar a abundância de dolicopodideos em diferentes espécies de adubos verdes. Para isso, foram utilizados os seguintes tratamentos: crotalária (Crotalaria spectabilis), feijão-caupi (Vigna unguiculata), girassol (Helianthus annuus), mamona (Ricinus communis) e trigo mourisco (Fagopyrum esculentum). A pesquisa foi realizada em campo, durante os anos 2012/2013 (novembro/dezembro a março) e 2013/2014 (outubro/novembro a fevereiro). As avaliações foram realizadas semanalmente, e a abundância de Dolichopodidae foi monitorada com uso de rede de varredura e instalação de armadilhas Moericke. Adicionalmente, foi quantificada a infestação de insetos fitófagos (Thysanoptera: Thripidae; Hemiptera: Aleyrodidae e Hemiptera: Aphididae), para avaliar possível relação de denso-dependência. No total, foram capturados 13987 dolicopodídeos (96,5% em armadilha Moericke), representados pela espécie Condylostylus erectus Becker, 1922 (Diptera: Dolichopodidae). De modo geral, a maior abundância de C. erectus foi observada em trigo mourisco e feijão-caupi. Em relação aos fitófagos, foram quantificados 48371 indivíduos (Thripidae (77,5%), Aleyrodidae (20,2%) e Aphididae (2,5%)), e não foi evidenciada uma consistente correlação populacional com C. erectus. Desse modo, esses resultados sugerem que o trigo mourisco e o feijão-caupi disponibilizam recursos alimentares de interesse ao predador, conforme discutido no trabalho. Por fim, a semeadura de trigo mourisco e feijão-caupi são opções para incrementar a abundância do predador C. erectus no agroecossistema.*
Palavras-chave: *controle biológico conservativo, manejo ambiental, biodiversidade, inimigos naturais, plantas atrativas.*

INTRODUCTION

Dolichopodidae family (long-legged flies) stands out for being constituted by several predatory species (ULRICH, 2004; BICKEL, 2009). These biological control agents feed on a range of arthropods (ULRICH, 2004), which characterizes their importance in agroecosystems (BOONE et al., 2008; LUNDGREN et al., 2014; CICERO et al.,2017). Although, this family is still poorly studied, its occurrence has been reported worldwide (BICKEL,2015; BICKEL et al., 2016; REZAEI ET al, 2019; NEGROBOV et al., 2020; RUNYON, 2020).

Considering the most recent studies in the world, 268 genera and 7,358 species have been reported (PAPE et al., 2011), while in Brazil, 31 genera

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and 212 species have been reported (CAPELLARI, 2017; CAPELLARI, 2020). Although, knowledge of the species is essential, there are still only few studies in Brazil that have investigated them in the field (BORTOLOTTO et al., 2016; HARTERREITEN-SOUZA et al., 2020). In the existing literature, it is possible to observe the importance of long-legged flies on aphids (MIRANDA et al., 1998), whiteflies (LUNDGREN et al., 2014), and other small arthropods of agricultural importance (KAUTZ & GARDINER, 2019; HARTERREITEN-SOUZA et al., 2020). Although, further studies are needed, it was observed that a long-legged fly can feed on one aphid per minute in a peach orchard (RATHMAN et al., 1988), indicating their ability to reduce the pest population. For this reason, it is important to know conservative strategies that allow the preservation and maintenance of these predators in the agroecosystem, so that they can assist in the biological control of pests.

In this regard, some studies have investigated different techniques for landscape preservation and manipulation of the community of natural enemies (HEIMPEL & MILLS, 2017; HAAN et al., 2019; GONZÁLES et al., 2020; CLOYD et al., 2020). Among the techniques used, the sowing of plants that provide food resources (e.g., floral resources such as pollen and nectar, in addition to the presence of prey or alternative hosts) has been strongly recommended (BARBOSA, 1998; SNYDER, 2019; MARTINEZ et al., 2020).

Regarding the Dolichopodidae family, there is no consensus in the existing literature regarding visitation behavior to plants with floral resources (KEVAN & BAKER, 1983; JAMES et al., 2014; PAIVA et al., 2018), suggesting that this behavior can vary according to the predator taxonomic level. Although, several plants may present resources of interest to natural enemies, cover crops have recently stood out (LUO et al., 2019; SOUZA et al., 2019), indicating their potential to be an essential tool in integrated pest management. The use of cover crops in agriculture has another benefit due to its importance in improving the physico-chemical properties of the soil (JUNIOR-ARAÚJO et al., 2015). Thus, they are plants that play an important ecological role, and can improve the population of natural enemies.

In spite of this, it is important to note that in Brazil there are no studies to date to understand the relationship between long-legged flies and cover crops. This information can lead to the accomplishment of complementary research that can make the recommendations of strategies of conservative biological control possible. Thus, the present research evaluated the abundance of Dolichopodidae in different cover crops species.

MATERIALS AND METHODS

Study location

The experiment was carried out in an experimental area located in Londrina city (23º 20’30’’ S, 51º12’49’’ W, 579 m above sea level), Parana State, during the years 2012/2013 (November/ December to March) and 2013/2014 (October/ November to February).

Climate

According to the Köeppen classification, the local climate is characterized as of the humid subtropical type (Cfa). The average temperature and precipitation (accumulated) during the study were respectively 27.51°C and 671 mm in the first year (2012/2013) and 29.5°C and 197 mm in the second year (2013/2014). The data were obtained from the weather station of Parana Agronomic Institute (IAPAR), Londrina.

Cover crops sown

In the study, the following cover crop species were compared: cowpea (*Vigna unguiculata*) (BRS Novaera), sunflower (*Helianthus annuus*) (unknown cultivar), castor bean (*Ricinus communis*) (cultivar BRS Energia), buckwheat (*Fagopyrum esculentum*) (cultivar IPR 92), and crotalaria (*Crotalaria spectabilis*) (unknown cultivar). To allow synchrony of the plant development stages, sowing was carried out in steps, in dates ranging between November 14 and December 5 (2012), and October 18 and November 23 (2013). Thus, in the first year (2012/2013), the evaluations were carried out between January and March, while in the second year (2013/2014) it varied between December and February.

The plots consisted of an area of 10 m^2 (2 m × 5 m in width and length, respectively), where six lines of each plant species were sown (0.3 m spacing rows). The only exception was of castor bean (four lines spaced at 0.5 m) due to a greater required for its development.

Experimental design

The experimental design was used in randomized blocks using five treatments (cover crop species) and five replications. A distance of 5 m was maintained between the plots to reduce the influence between treatments. The blocks were represented by strips of plants, positioned between the soybean and corn fields, east and west, respectively.

Dolichopodidae assessment on cover crops

The evaluations were carried out in the vegetative and reproductive phenological stages. In the first (2012/2013) and second year (2013/2014), respectively, the number of evaluations in the vegetative (V) and reproductive (R) stage in each species were: cowpea (5V and 2R; 5V and 2R), sunflower (2V and 5R; 2V and 5R), buckwheat (2V and 5R; 1V and 6R), castor (2V and 5R; 2V and 5R), and crotalaria (5V and 2R; 5V and 2R).

Dolichopodidae quantification was carried out using two sampling methods: sweep net (23 cm in diameter) method (NOURTI et al., 2019) and the Moericke trap method (MARCHIORI et al., 2016). For net sampling, each treatment were "swept" weekly 10 times (n = 50 evaluations/treatment), between 10:00 a.m and 12:00 p.m (time of greatest insect activity). The specimens collected were placed in plastic bags previously moistened with ethyl acetate to kill the insects. Subsequently, the insects were screened in the laboratory.

Moericke traps were also checked weekly. This trap consisted of water (around 200 mL), detergent (1%) (to break water surface tension), and formaldehyde (1%) (for insect preservation). The traps (n = 2/plot) were evenly positioned on a support at the center of the plots and were adjusted to the height of the canopy of the plants, according to the plant's growth. Moericke traps remained in the field for 48 h (each assessment). After collecting the material, the insects were screened and long-legged flies were sent to the Diptera Morphology and Evolution Laboratory, at UNESP, to identify the species.

Sampling of phytophagous insects associated with cover crops

To assess the density-dependency relationship, possible prey of Dolichopodidae, such as thrips (Thysanoptera: Thripidae), whiteflies (Hemiptera: Aleyrodidae), and aphids (Hemiptera: Aphididae) (ULRICH, 2004), were assessed. These insects were captured using the same methodology as that for Dolichopodidae assessment. The insects captured were sent to the laboratory and placed in 70% alcohol. Subsequently, they were identified (to the family level) using a stereoscopic microscope.

Statistical analysis

The results obtained were submitted to exploratory analyses to evaluate the assumptions of normality and residuals independence, variance homogeneity and the additivity of the model to allow the application of the analysis of variance (ANOVA). Multiple comparisons of means were performed using the Tukey test. For non-parametric data, square root transformation (x + 0.1) was performed. To assess density-dependency relationship, cover crops with the highest long-legged fly capture were selected and Pearson's correlation analysis (r) was performed. Finally, the correlation test (r) was used to evaluate the rain effect of rain on Dolichopodidae population. For all analyses, a value of $P \leq 0.05$ was used to test the significance of the results.

RESULTS

Dolichopodidae abundance (%) in cover crops

In total, 13,987 individuals were captured, represented only by *Condylostylus erectus* Becker, 1922 (Diptera: Dolichopodidae). Interestingly, it was observed that in the second year of the study (2012/2013), approximately twice as many (67.45%) predators were captured as that in the first year (32.55%).

Regarding the sampling method, Moericke traps captured 96.5% (N = 13,500 specimens), while 3.5% was captured in sweep nets (N = 487 specimens). In general, the highest number of captures were observed on cowpea and buckwheat (Table 1). Using the sweep net, cowpea showed higher Dolichopodidae population, however, this sampling method was less representative.

Phytophagous abundance (%) in cover crops

In total, 48,371 insects were captured. Similar to Dolichopodidae, the highest phytophagous abundance (relative) was observed in Moericke traps (71%) (Table 2). Among the insect families, the most abundant was Thripidae (77.2%), followed by Aleyrodidae (20.3%). The Aphididae family accounted for only 2.5% of the total. Additionally, a slightly lower (45%) infestation was reported in the first year of study (2012/2013) than in the second year (55%) (2013/2014).

In general, the Moericke traps indicated higher Thripidae occurrence in cowpea (2012/1013 and 2013/2014) (Table 2). However, this was not observed in the sweep nets (Table 3). The families Aleyrodidae and Aphididae were not significantly influenced by the cover crop species (Tables 2 and 3).

Biotic (Dolichopodidae and insect prey) and abiotic (rain) correlation

In general, there was no correlation between Dolichopodidae population and populations

Table 1 - Dolichopodidae (mean ± SE) adults captured in cover crop using sweep net and Moericke trap. Londrina, Paraná State, 2012/2013 (November to February; N = 7 assessments) and 2013/1014 (December to March; N = 7 assessments).

Cover crop	-------------------------Sampling---------------------		-------------------------Sampling--------------------------	
	Sweep net		Moericke trap	
	2012/2013	2013/2014	2012/2013	2013/2014
Cowpea	9.4 ± 1.69 a	53.6 ± 10.70 a	256 ± 38.52 ab	429 ± 80.83 ab
Sunflower	0.2 ± 0.2 cb	1.4 ± 0.50 b	134 ± 37.91 c	156 ± 12.50 c
Buckwheat	2.2 ± 0.37 b	14.6 ± 6.24 b	328.2 ± 65.04 a	577.6 ± 67.66 a
Castor bean	3 ± 0.44 b	7.8 ± 0.96 b	24.4 ± 8.27 d	296.2 ± 42.81 bc
Crotalaria	2.6 ± 0.4 b	3.4 ± 0.74 b	189 ± 42.57 bc	362.4 ± 52.73 b
CV (%)	20.39	32.50	11.41	26.31
GL error	16	16	16	16
F	24.24	17.76	62.43	13.29
p	<0.01	<0.01	<0.01	<0.01

[1]Means ± SE followed by the same letter in the column do not differ by the Tukey test (5% probability). [2]Original results followed by the analysis carried out with the data transformed into arcsen √ (x / 100).

of Thripidae and Aleyrodidae families (Table 4). The Aphididae family population showed a significant relationship with the predator family population only in buckwheat (Table 4).

Although, the predators capture was higher (67.45%) in the least rainy year (2013/2014), there was no influence of rain on the Dolichopodidae population fluctuation [(r =

Table 2 - Phytophagous (mean ± SE) captured in cover crop using sweep net. Londrina, Paraná State, 2012/2013 (November to February; N = 7 assessments) and 2013/1014 (December to March; N = 7 assessments).

Cover crop	--------------------------------------Phytophagous families (2012/2013)--------------------------------------		
	Thripidae	Aleyrodidae	Aphididae
Cowpea	67.6 ± 8.30 c	17.02 ± 5.07 b	4.2 ± 2.96 ab
Sunflower	20.6 ± 5.52 c	6.4 ± 2.48 c	1.2 ± 0.73 bc
Buckwheat	194.6 ± 34.01 b	25.08 ± 5.56 b	4.6 ± 0.36 a
Castor bean	31 ± 8.52 c	8.2 ± 2.42 c	0.4 ± 0.36 c
Crotalaria	248.6 ± 27.02 a	45.2 ± 8.54 a	2 ± 1.25 abc
CV (%)	23.28	41.50	36.80
GL error	16	16	16
F	77.23	17.15	7.46
p	<0.01	<0.01	<0.01
Cover crop	------------------------------------Phytophagous families (2013/2014)--		
	Thripidae [2]	Aleyrodidae [2]	Aphididae
Cowpea	348.8 ± 38.89 b	4.2 ± 0.55 a	12.2 ± 8.97 b
Sunflower	167 ± 41.79 c	1.8 ± 0.99 a	1.6 ± 1.01 b
Buckwheat	956.6 ± 146.12 a	2.6 ± 1.30 a	33.6 ± 10.53 a
Castor bean	172.8 ± 28.10 c	4.2 ± 0.55 a	2.6 ± 0.89 b
Crotalaria	361.4 ± 41.08 b	3.8 ± 1.37 a	3.8 ± 2.28 b
CV (%)	55.95	49.58	58.45
GL error	16	16	16
F	11.48	2.12	8.97
p	<0.01	ns	<0.01

[1]Means ± SE followed by the same letter in the column do not differ by the Tukey test (5% probability). [2]Original results followed by the analysis carried out with the data transformed into arcsen √ (x / 100). * Non-significant ANOVA.

Table 3 - Phytophagous (mean ± SE) captured in cover crop using a Moericke trap. Londrina, Paraná State, 2012/2013 (November to February; N = 7 assessments) and 2013/1014 (December to March; N = 7 assessments).

Cover crop	--Phytophagous families (2012/2013)--		
	Thripidae	Aleyrodidae	Aphididae
Cowpea	791.6 ± 193.71 a	338.8 ± 126.41abc	21.6 ± 4.55 a
Sunflower	100 ± 42.05 c	507.4 ± 144.81 a	4.4 ± 1.86 b
Buckwheat	486.8 ± 117.92 b	481.4 ± 118.75 ab	7.6 ± 1.86 b
Castor bean	143 ± 66.01 c	288 ± 51.11 bc	5.6 ± 1.74 b
Crotalaria	489.6 ± 114.98 b	216.6 ± 35.76 c	10.6 ± 3.91 b
CV (%)	32.18	27.55	35.77
GL error	16	16	16
F	24.24	7.73	18.85
p	<0.01	<0.01	<0.01
Cover crop	--Phytophagous families (2013/2014)--		
	Thripidae [2]	Aleyrodidae	Aphididae [2]
Cowpea	1026.4 ± 93.71 a	19.6 ± 2.39 b	12.2 ± 2.08 b
Sunflower	300.6 ± 98.94 b	28.2 ± 6.80 ab	15.4 ± 16.37 b
Buckwheat	488.6 ± 131.79 b	48.8 ± 14.72 a	49.6 ± 17.93 a
Castor bean	558 ± 157.47 b	28.6 ± 4.72 ab	22.4 ± 11.32 ab
Crotalaria	570 ± 70.58 b	18.4 ± 1.38 b	7.6 ± 2.24 b
CV (%)	29.55	28.65	40.21
GL error	16	16	16
F	11.80	5.05	4.91
p	<0.01	<0.01	<0.01

[1]Means ± SE followed by the same letter in the column do not differ by the Tukey test (5% probability). [2]Original results followed by the analysis carried out with the data transformed into arcsen √ (x / 100). [*]Non-significant ANOVA.

−0.18; P = 0.69) 2012/2013 and (r = 0.065; P = 0.88) 2013/2014].

DISCUSSION

The present study demonstrated for the first time in Brazil that cover crops such as cowpea and buckwheat are important plants to improve *C. erectus* populations in agroecosystems. Thus, the management of these cover crops is an important tool for conservative biological control. Although, the relationship between cover crops and natural enemies is widely studied (DAMIEN et al., 2017; MARTINEZ et al., 2020), in Brazil this is the first report associated with the Dolichopodidae family.

The importance of the genus *Condylostilus* in the Brazilian agroecosystem has already been previously reported, however, from the Federal District region (HARTERREITEN-SOUZA et al., 2014; HARTERREITEN-SOUZA et al., 2020). These authors observed *C. erectus* species predominantly in agricultural areas, probably due to the higher prey abundance. This information is important, as it allows us to infer that cover crops such as cowpea and buckwheat may provide alternative food (pollen and/or nectar) for these predators when prey availability is low in field crops; recolonizing cultivated areas when the prey population increases (HARTERREITEN-SOUZA et al., 2020). In the existing literature, some studies have reported the predation ability of the Dolichopodidae family. For example, a study from a peach orchard evidenced that *Medetera petulca* Wheeler, 1899 (Diptera: Dolichopodidae) had a predation potential to feed on one aphid per minute (RATHMAN et al., 1988). Although, there are no similar studies for *Condylostilus*, it was observed that this genus has the capacity to affect the aphid population in wheat crops by approximately 50% (BORTOLOTTO et al., 2016), indicating an important biological control potential. Therefore, based on evidences of aphid predation, it is suggested that cowpea and buckwheat strips can be probably more successful if wheat fields are adopted in the neighboring areas to reduce aphid infestation.

Although, it was not the objective of the study, Moericke traps were highly efficient

Table 4 - Correlation between phytophagous insects abundance and Dolichopodidae in cover crop (using a Moericke trap; N total = 14 evaluations). Londrina, Paraná State, 2012/2013 (November to February; N = 7 assessments) and 2013/1014 (December to March; N = 7 assessments).

Cover crop					
Cowpea			Buckwheat		
Thripidae	Aleyrodidae	Aphididae	Thripidae	Aleyrodidae	Aphididae
r	r	r	r	r	r
0.2096	-0.0283	0.1429	0.0624	0.0245	0.6541
p	*p*	*p*	*p*	*p*	*p*
0.0815	0.8163	0.2377	0.6077	0.8407	<0.0001

Pearson's correlation test (r); $P < 0.05$.

in capturing *C. erectus*, in comparison with the sweep nets. This difference can be explained by two possibilities. The first is that the agility of the long-legged fly agility (SOURAKOV, 2011) makes it difficult to capture using a net. The second is that the yellow color of the Moericke trap is highly attractive to Dolichopodidae (HOBACK et al., 1999). Additionally, it is important to note that Moericke traps remained for a period of two days in the field, enabling a greater capture of long-legged flies.

An interesting observation at study was the significant variation of Dolichopodidae abundance between the two years. For example, the capture in the first year (2012/2013) was approximately only a third of that in the second year. This contrast in the captured population can be explained by heavy rain in the first year (2012/2013), which may have reduced the predator's abundance. For example, in the first year there were nine days that were relatively rainier (above 30 mm/day), while in the second year there were only three days with these conditions (above 30 mm day). Thus, it is likely that the higher soil moisture may have caused higher larval mortality of Dolichopodidae. This hypothesis is supported by the fact that during the young phase, which occurs in the soil, these insects are highly susceptible to heavy rain (FROUZ, 1999). Conversely, at the adult stage, Dolichopodidae family is less affected by rain and can reproduce normally (FROUZ, 1999). This behavior is interesting, since in the present study there was no relationship between rain and adult Dolichopodidae population fluctuation, as shown in previous studies.

Interestingly, in the present study the abundance of *C. erectus* population was not correlated with that of phytophagous insects in cover crops, indicating the absence of density-dependency. This result is in contrast with the findings of BORTOLOTTO et al. (2016) study, in which the authors verified a significant Dolichopodidae influence on aphids infestation in wheat fields. However, this can be justified by the difference in the availability of resources that the plants present. For example, buckwheat assessment occurred mostly (approximately 80%) in flowering stage, suggesting that flowers provide important food resources to *C. erectus*. Although, the importance of this cover crop for natural enemies is already known (JAMES et al., 2014; PENCA et al., 2017; FOTI et al., 2017), the floral visitation habit is not common across the Dolichopodidae family (LARSSON et al., 2001; JAMES et al. 2014; PAIVA et al., 2018), probably due the taxonomic level variability. In this sense, the findings of the present study suggested that floral resources (pollen and/or nectar) of buckwheat are important for *C. erectus* species. However; although, buckwheat inflorescence may increase the diversity of natural enemies (FOTI et al., 2017), this relationship needs to be tested in additional laboratory studies to confirm the hypothesis.

Regarding cowpea, unlike buckwheat, most evaluations occurred in vegetative stage (approximately 66%). This observation is important, as cowpea is characterized by presenting extra-floral nectaries on the plant's branches (JONES et al., 2017). Due to this distinct morphological characteristic, cowpea can provide complementary food (carbohydrates) to natural enemies (ZHU et al., 2014) during practically the entire development cycle. The importance of carbohydrates to Dolichopodidae has already been shown by a previous study (KOST & HEIL, 2005); it was observed that the application of sugar solution on lima beans (*Phaseolus lunatus*) leaves increased the Dolichopodidae population. Thus, the occurrence of *C. erectus* in cowpea is probably associated with the extra-floral nectar availability for the predator. This hypothesis is reinforced by the lack of relationship between *C. erectus* and the phytophagous infestation in the plant. However, it is necessary to emphasize that; although , floral resources and extra-floral nectaries may be important (MATHEWS et al., 2009), there are other plant characteristics that must be considered. For example, in this study, among the plants with lower Dolichopodidae capture were castor bean and sunflower, and both species show extra-floral nectaries. This difference may be due to to the tall height of the plants, which causes a microclimate unfavorable to predators, such as greater sunlight exposure and lesser humidity (VILKS, 2007). However, despite the lower Dolichopodidae capture in these plants, castor beans and sunflower can offer food subsidy to other natural enemies that occur freely in agroecosystems (BASIT et al., 2016; LÓPEZ-GUILL ÉN et al., 2020).

Finally, the present study demonstrated for the first time in Brazil that buckwheat and cowpea are alternatives for increasing the population of *C. erectus* in agroecosystems. This information is basic and may provide support for carrying out complementary studies in the field, investigating the effect of sowing these cover crops on the target pests (particularly aphids) of cultivated plants.

CONCLUSION

Buckwheat and cowpea cover crops increase *C. erectus* predator populations inagroecosystems.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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