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EFFICIENCY OF *Stethorus sp* (COLEOPTERA: COCCINELLIDAE) IN THE CONTROL OF *RAOIELLA INDICA* (ACARI: TENUIPALPIDAE)

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Abstract: *Cocos nucifera* L. assumes great importance in Brazilian agriculture. The crop faces several phytosanitary problems, among which those caused by phytophagous mites stand out. Concerns have arisen about the consequences of establishing *Raoiella indica* Hirst, where plants of economic and ecological importance can be potentially affected, considering that this pest mite causes significant damage to coconut, banana and ornamental palm trees. Since it was reported in Brazil, studies have been carried out to combat the pest. The use of different acaricides has been the main way of combating this phytophagous mite. However, efforts have become expensive, considering the wide variety of host plants and the high potential for pest dispersion. In this sense, the use of natural enemies has shown promise in the management of *R. indica*. The objective of this work was to evaluate the potential of *Stethorus* sp in the control of *R. indica*. The experiments were carried out in the Acarology Laboratory of the Department of Entomology and Acarology of the Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ/USP). Nine populations of *Stethorus* sp from different Brazilian regions. The population that showed the highest rates of predation and oviposition was collected in the state of Maranhão and the one with the lowest potential was that collected in the state of Minas Gerais, taking into account all life stages (eggs, larvae, adults) of the species. pest mite offered as food to the predator. This study contributed to the advancement of knowledge about the predatory mite and to the establishment of biological control programs for *R. indica* in coconut cultivation, in the different Brazilian regions where the pest is present.

Keywords: Red palm mite, biological control, predatory insects, functional response.

INTRODUCTION

The coconut (*Cocos nucifera* L.) assumes great importance in Brazilian agriculture, as it generates employment and income throughout the entire production chain in which it is inserted (Fontes; Mucio, 2006; IBGE, 2019). In recent years there has been a significant increase in planted areas, as well as in the percentage of production and productivity. Among other factors, the use of more productive cultivars, the adoption of new cultivation technologies and the greater proximity to large consumer centers are attributed (Fontes et al. 2003).

The expansion of the planted area in the different regions of the country, as well as the movement of seedlings from one region to another, brought gains. However, the aggravation and occurrence of phytosanitary problems in the coconut crop began to be observed (Navia, 2008, Barroso et al. 2019).

One of the major obstacles to coconut farming has been the attack of pests, including phytophagous mites. Several species can cause severe symptoms to plants, making it necessary to apply control measures to reduce damage. Among the phytophagous mites, *Raoiella indica* Hirst has caused concern for coconut producers in Brazil, since its first registration in the country in 2009.

Its main host is the coconut tree, which can reach very high population densities and cause significant damage to plants (Peña et al. 2009; Barros et al. 2020). Infestations found on other host plants are minor but equally problematic due to their importance as native and/or ornamental species (Carrillo et al. 2012a).

Infestations typically occur on the abaxial surface of leaves causing the appearance of yellowish spots that soon progress to necrotic spots (Etienne; Flechtmann, 2006; Rodrigues et al. 2007). In severe infestations, the death of young plants has been observed, as well as the

drop of flowers and fruits during the fruiting period, causing a reduction in the number of fruits produced. (Welbourn, 2007; Peña et al. 2006).

The use of different acaricides has been the main way to combat *R. indica* in coconut trees. However, the use of these products for the management of this pest becomes difficult and expensive due to the variety of host plants and the dispersion of *R. indica* in natural, agricultural, recreational and residential areas (Peña et al. 2007; Rodrigues; Peña, 2012). Added to this is the mite's high reproductive potential, as well as the tall size of older plants, which makes it difficult to access the palm tree canopies for the application of chemical products (Mendonça et al. 2005; Peña et al. 2007).

Currently there is a great interest in implementing alternatives to the management of this pest. Among the control methods, the use of natural enemies has shown promise in the management of *R. indica*. In this sense, biological control has been seen as a possible alternative considering that there are numerous natural enemies associated with *R. indica* (Carrillo et al., 2012a).

Some predatory insect species belonging to the Coccinellidae family have been reported in association with *R. indica* (Houck, 1991; Ullah, 2000; Bibbinger et al. 2009). Species belonging to the genus: *Stethorus* have been reported in association with the pest and demonstrate a high potential for predation. Among these: *Stethorus keralicus* Kapur (Puttaswamy; Rangaswamy, 1976; Daniel, 1981; Hoy et al. 2006); *Stethorus utilis* Horn (Peña et al. 2009); *Stethorus tetranychii* Kapur e *Stethorus parcempunctatusi* (Daniel, 1981; Gupta, 2001); *Stethorus pauperculus* Weise (Yadavbabu; Manjunatha, 2007), *Stethorus aptus* Kapur (Govindasamy et al. 2018) have been frequently cited as effective predators of *R. indica*.

Although the potential of these species for biological control is known, they remain little explored as a biological control agent for *R. indica*. Further studies on these species reported in association with *R. indica* are necessary as they can represent an important tool in the management of this invasive species in the Neotropics and in other regions of the world (Carrillo et al., 2012b). Therefore, the objective of the present study was to evaluate the potential use of different populations of *Stethorus sp* in control of *Raoiella indica*.

MATERIAL AND METHODS

COLLECTIONS OF THE POPULATIONS OF *Stethorus sp* IN DIFFERENT BRAZILIAN REGIONS

The collections were carried out in six Brazilian states in different cities, covering four regions of the country. The collections took place between February 2020 and June 2021. The populations were collected in the states of Ceará, Maranhão, Minas Gerais, Rio de Janeiro and Rio Grande From north. The beetle collections were carried out in cabbage plants, as well as in young coconut palm plants, making cloth beats on the leaves inside plastic trays, then suctioning the insect using a suction tube coupled to a pipette tip.

ESTABLISHMENT OF THE POPULATION OF *Stethorus sp* UNDER LABORATORY CONDITIONS

Colony maintenance and experiments were carried out at the Acarology Laboratory of the Department of Entomology and Acarology of the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ/USP).

Initially, the individuals were kept in experimental units (plastic trays 16 x 22 x 7 cm) with pieces of polyethylene foam at their base (2 cm high) and sheets of *Canavalia ensiformis* (pork beans) and inside it, distilled water is added to keep the leaves moist. As food

was offered: *R. indica* and *Tetranychus urticae* (all developmental stages), as a natural food source, as well as alternative food such as eggs *Anagasta kuehniela* (Lepidoptera: Pyralidae). An artificial diet based on brewer's yeast and honey in a 3:1 ratio was also provided.

As soon as oviposition occurred, the eggs were separated from the adults, to other trays to prevent them from being preyed on by the adults and thus allowing the hatching of the larvae. Newly hatched larvae, originating from eggs of females from the maintenance breeding, were individualized in glass tubes (8.0 cm x 2.2 cm). An artificial diet based on brewer's yeast and honey in a 3:1 pasty consistency ratio was also provided to feed the larvae. The recipients with the coccinellids were kept in a BOD-type climatized chamber, at 25.0 ± 1 °C, $70.0 \pm 10\%$ RH and 12 h of photophase. Tests were conducted using adults of the predator.

Adult predators were transferred to cabbage plants, produced in 3-liter pots, with 2 plants in each pot. The pots were arranged in a screened cage measuring 80 x 110 x 50 cm placed on a bench inside the breeding room. Five couples of the predator were transferred to each pot, and coconut leaflets containing *R. indica*, *T. urticae* and container containing eggs *A. kuehniela* and another container with an artificial diet composed of honey and brewer's yeast of pasty consistency, which served as a complementary food for the adults.

ASSESSMENT OF THE PREDATION POTENTIAL OF *Stethorus* sp OVER *RAOIELLA INDICA*

To conduct this stage of the experiment, 25 females of the predator were individualized. Each experimental unit consisted of a small Petri dish (2 cm high x 3 cm in diameter) whose base was covered with a layer of carrageenan, superimposed by a disk of jack bean leaf (2 cm in diameter). The carrageenan

layer was prepared by dissolving about 10.0 g of carrageenan in 70 ml of distilled water (at 80-90°C), heated for 30 seconds in a microwave oven. This amount was sufficient to prepare 25 experimental units.

Sixty eggs of *R. indica* were placed in each arena and an adult female of *Stethorus* was transferred to each unit. In total, 25 females were evaluated at this stage of the experiment. For the larval test, sixty phytophagous mite larvae were placed in each arena with an adult female of the predator individualized in the experimental unit. In total, 25 females were evaluated in this treatment. Also, sixty adults from *R. indica* were placed in each arena and an adult female of *Stethorus* was transferred to each unit. Twenty-five females were evaluated for this treatment. The treatments were the prey development stages, *R. indica* [Treatments: 01) eggs; 02) larvae; 03) adults].

The experimental units were sealed with plastic film (magipack®) to prevent predators from escaping. They were kept in an acclimatized chamber, at 25.0 ± 1 °C, $70.0 \pm 10\%$ RH and 12 h of photophase. The study was performed for 7 consecutive days, with an assessment every 24 h performed under a stereomicroscope. The variables evaluated were the daily oviposition of the predator and the number of prey consumed per female per day. The results of the first day were discarded, to reduce interference from the previous feeding. At the end of each daily evaluation, the prey consumed (eggs, larvae or adults) was replaced, maintaining the initial amount. The predator's eggs were also taken from the arenas daily.

The procedures described above were carried out in different periods for all predator populations collected in the field, evaluating the predation potential on the prey development stages (egg, larva and adult), as well as the oviposition of the predatory insect.

STATISTICAL ANALYSIS

Predation data were submitted to a mixed generalized linear model with binomial distribution. While the mean egg laying data were analyzed with a mixed generalized linear model with Poisson type distribution. Both models were programmed using the glmer function of the lme4 package (Bates et al. 2015) of R (R Core Team, 2020), the population factor was considered as a random effect, while the mite stage was considered as a fixed effect in the model. The fit of the model to the data was confirmed by means of a simulated half-normal envelope of R (R CORE TEAM, 2020). The data predicted by the model were obtained through a bootstrap based on mixed models with the bootMer function of the lme4 package (BATES et al., 2015) of R (R CORE TEAM, 2020).

RESULTS

ASSESSMENT OF THE PREDATION POTENTIAL OF *Stethorus sp* OVER *RAOIELLA INDICA*

In view of the observed results, it was possible to show that the egg stage was significantly more predated in relation to the immature and adult stages, regardless of the population. On the other hand, in the CE and RJ populations, no significant differences in predation were observed between the egg and immature stages, while for the other populations, the immature stages were significantly more preyed upon than the adults (Table 1.2).

There was a statistically significant difference in oviposition rates between the different populations of *Stethorus sp* evaluated. The highest oviposition rates were observed for the population from Maranhão, considering all the populations of *Stethorus sp*, evaluated and all stages of the pest mite provided as food. The lowest oviposition rates were recorded for the population collected in

the state of Pará, taking into account all stages of the pest evaluated (Table 3).

For most populations, no significant differences were observed in oviposition rates for the different life stages of *R. indica* used as food for the predatory mite. Only for the population of MG there was an influence of the stage of *R. indica* about the oviposition of *Stethorus*, with lower oviposition rate when predators were fed (immature) larvae of the pest mite.

DISCUSSION

The results of the present study suggest that populations of *Stethorus*, originating in several Brazilian regions, present differences in the predation potential of *R. indica*, when compared to each other. These differences may be associated with several factors such as genetic variability between lineages/populations of the predatory insect regarding the predation potential, reproduction and history of exposure of the predator to prey, as well as the origin (geographic regions with different climatic conditions) of the population of the predator (Carrillo et al. 2012b, Domingos et al. 2013, Gomes-Moya et al. 2018).

Of the regions where the insects were collected, Minas Gerais was the state with the lowest density of *R. indica* in coconut plants. And the predators of this region showed the lowest predation potential when compared to the other populations. It hasn't been long since the mite: *R. indica* was detected in this region, suggesting that the predator has little time of exposure to the prey.

The interesting features of *Stethorus* used in biological control to suppress the population of phytophagous mites are prey consumption, longevity and high reproductive capacity (Bibinger et al. 2009).

The adult stage of *R. indica* was the one with the least predation by *Stethorus* when

Population	Egg			Immature (larva)			Adult		
CE	0,6100	a	C	0,3100	b	C	0,2300	c	B
MA	0,9400	a	A	0,3800	b	A	0,2000	c	A
MG	0,5000	a	D	0,1500	b	D	0,1400	b	B
MT	0,7000	a	B	0,1700	b	D	0,2300	c	B
RJ	0,6400	a	C	0,3000	b	C	0,2500	c	B
RN	0,6000	a	C	0,3000	b	C	0,2300	c	B

Table 1. Predation rate of stages of *Raoiella indica* em populations of *Stethorus sp*, from different states of Brazil.

Means followed by the same lowercase letters (comparison between columns) and uppercase letters (comparison between rows) do not differ by overlapping confidence intervals (95%CI) estimated by the generalized linear model with binomial distribution.

Population	Egg			Immature (larva)			Adult		
CE	60,6	a	C	26,6	b	C	22,8	c	B
MA	90,4	a	A	30,8	b	A	26	c	A
MG	34,0	a	D	33,0	b	D	22,4	b	B
MT	76,0	a	B	44,2	b	D	31,8	c	B
RJ	52,4	a	C	36,0	b	C	21,8	c	B
RN	50,0	a	C	38,0	b	C	23,8	c	B

Table 2. Daily average of predation stages of the *Raoiella indica* by populations *Stethorus sp* from different states of Brazil.

Means followed by the same lowercase letters (comparison between columns) and uppercase letters (comparison between rows) do not differ by overlapping confidence intervals (95%CI) estimated by the generalized linear model with binomial distribution.

Population	Egg			Immature			Adult		
CE	1,96	A	B	0,88	A	C	1,07	A	BC
MA	2,53	A	A	1,84	A	A	2,02	A	A
MG	2,90	A	B	0,62	A	C	0,97	A	C
MT	2,01	A	B	1,15	A	BC	2,14	A	B
RJ	2,00	A	B	1,19	A	B	2,25	A	B
RN	2,37	A	A	1,25	A	B	2,32	A	B

Table 3. Daily average of eggs from populations of *Stethorus sp* coming from different states of Brazil, supplying themselves to different stages of *Raoiella indica* as a food.

Means followed by the same lowercase letters (comparison between columns) and uppercase letters (comparison between rows) do not differ by overlapping confidence intervals (95%CI) estimated by the generalized linear model with Poisson distribution.

compared to eggs and larvae. This fact may be related to the presence of glandular setae that eliminate secretion (Di Palma et al. 2021) and that these substances may have a repellent action, serving to repel the predator. This decreasing consumption in the proportion of prey consumption may also be related to the level of satiety and the handling time required for the prey to be consumed (Britto et al. 2008).

Several studies have been carried out to evaluate the feeding capacity of different species of *Stethorus*, but most of them use only prey eggs or all stages together, which makes it difficult to know which prey stages are most attractive to the predator (Fiaboe et al. 2007). In our study, we separated the stages of *R. indicica* and we can observe that the most preferred stage was the egg stage. However, the preferred prey stage may vary according to the species and developmental stage of the predator (Ragkou et al. 2004).

The use of predatory insects as pest control agents, whether these phytophagous mites has been intensified worldwide. Some species of predatory insects have been successfully used in different biological control strategies, whether by importation, augmentation and conservation (Fontes et al. 2020). Intensified research could lead to the discovery of a greater number of promising species that could even be more efficient than those already in use (Pena et al. 2009; McMurtry et al. 2013).

The results of this work are encouraging, demonstrating a high capacity for predation of *Stethorus sp* over *R. indicica* in laboratory. However, the contribution of *Stethorus sp* in the control of *R. indicica* in coconut plantations requires further studies involving ecological and biological aspects, especially its predatory potential and effects of acaricides or pesticides on the population of these insects.

These results justify the conduction of new studies under semi-field and field conditions, to

enable the use of this predator in the fight against the pest mite, by producers of coconut and other crops (eg: bananas, ornamental palms) attacked by *R. Indica*. Therefore, it is important to continue investing in studies to prospect new species of predatory insects, in the evaluation of their efficiency and in methods for their mass production and application, thus optimizing their use as a biological control agent.

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