Use of Basil *Ocimum basilicum and Chrysoperla externa* (Chrysopidae) in Agroecological Management of Rosebushes

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Abstract— *The intensive cultivation of flowers in a greenhouse often presents low diversity of plant species and this limits the preservation of natural enemies for pest control. Floral resources may provide multiple ecosystem services and promote regulation of pest populations in greenhouses. Chrysoperla externa (Chrysopidae) is a predator of various pests in the Neotropical region. The purpose was to evaluate the effect of basil (Ocimum basilicum) and C. externa releases on agroecological pest management in rosebushes and compare it with conventional management. The greenhouse with rosebush 'Carolla' was divided in two parts, one side with diversified rosebush (rosebush + basil + C. externa releases) and the other side with conventional rosebush (insecticides, acaricides and fungicides). Arthropods were sampled weekly in the rosebushes and basil. The abundance and diversity were different between the systems evaluated. Greater abundance of natural enemies and pollinators was observed in the diversified rosebush. Basil has attracted a greater diversity of natural enemies and had a positive effect on pest control in the rosebush. In conventional rosebushes most insects were phytophagous. The production and quality of roses were not influenced by the treatments. Agroecological pest management favors the biological control in rosebush cultivation in greenhouse.*

Keywords— Biological control, Floral resources, Habitat management, Natural enemies, Roses.

I. INTRODUCTION

Cultivation in the greenhouse has been extensively used by farmers and flowers and ornamental plants comprise about 650,000 ha in the world [1]. The estimate in Brazil is approximately 30,000 ha with greenhouses and the production of flowers represents about 10% of this area being the rose the most produced and commercialized cut flower [2].

One of the main issues associated with the loss of quality of flowers during cultivation refers to the pest arthropod infestation, affecting plant growth, flowering and causing aesthetic damage to floral buds. The most widely used pest control in flower production is still the chemical one, which has been causing problems of resistance and insecticide residues, besides affecting the populations of natural enemies and may contaminate workers and the environment [1,2,3].

The biological control of pests has been widely used in ornamental cultivation in greenhouses, which is mainly based on periodic releases of predators and parasitoids, that is, in the augmentative biological control [2,4]. Nevertheless, the effectiveness of this method may often be insufficient due to the difficulties of establishment and persistence of natural enemies in this environment [4,5], since intensive cultivation of flowers in a greenhouse usually presents low diversity of plant species, which limits the preservation of natural enemies for pest control.

Habitat management, through plant diversification, is a tool used to regulate pest populations in greenhouses. The conservative biological control can improve the establishment and maintenance of biological agents in the greenhouse. Studies have shown that more diversified agricultural systems can promote increased species richness and/or abundance of natural enemies of pests [5,6,7]. The use of flowering plants (floral resources) may promote the establishment and support of natural enemies' populations, with the aim of improving the control of pests such as aphids, thrips, among others [6,7,8,9,10,11]. Selecting the plant with adequate floral resource is an important factor to promote the attraction and accessibility of natural enemies in the cultivation area.

The predator *Chrysoperla externa* (Neuroptera: Chrysopidae) is an efficient pest controller of various pests such as aphids, lepidopterans, among others, with 100,000 to millions of individuals being commercialized per week in Latin America [2]. This species' adults are highly mobile, have a high reproduction rate and their larvae are voracious and have a great capacity for foraging [12]. In rosebush cultivation, research has been conducted aimed at the effective using of this predator as a biological control agent [10,13].

Basil (*Ocimum basilicum* L.) is an aromatic plant widely used in cooking and in the cosmetics and perfumery industry. Studies have shown basil being used as an attractive plant to different predators and parasitoids [11,14,15]. Agroecological pest management is aimed at promoting the balance of the system by reducing the pest population and increasing the population of beneficial insects. Thus, the purpose of this work was to assess the effect of basil as an attractive plant and of releases of *C. externa* in agroecological pest management on rosebushes in greenhouse and compare it with conventional management.

II. MATERIALS AND METHODS

The experiment was conducted from October to December 2016 in Minas Gerais, Brazil, at an altitude of 2917 ft. and geographical coordinates 21°06' S and 44°15' W. The local weather is mild (Cwa, according to the Köppen classification), which is characterized by humid summer and dry winter. The greenhouse (59 ft. x 19.7 ft.) was divided into two equal parts, transversely separated by a plastic (6.6 ft. high x 19.7 ft. long). On one side a diversified rosebush (agroecological management) (rosebush + basil + *C. externa* releases) was cultivated and on the other side rosebush with conventional management (insecticides, acaricides and fungicides). Each side of the greenhouse held 200 roses distributed in four flowerbeds with 50 plants per bed. We used 'Carolla' rose, which has a dark red color, velvety petals, and is well accepted in the market. The rosebushes were grown in single rows with spacing of 3.94 ft. between flowerbeds and 0.66 ft. between plants. The cultural treatments such as fertilization, irrigation, weeding, and pruning were carried out in both areas when necessary.

In the diversified rosebush cultivation, ten basil pots were planted on the sides of the greenhouse. Basil seedlings were planted in pots (2.6 gallons) containing soil and commercial substrate (Plantmax®) in a 2:1 ratio. Basil was used when in full bloom and approximately 19.68 in high. The second and third instar larvae of *C. externa* used in the releases were reared at 25±2ºC, relative humidity of 70±10% and photophase of 12 hours. Eggs up to 24 hours' old were individualized and after hatching the larvae were fed with eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae) until they reached the second and third instars to be used in the releases. The first release of *C. externa* was made seven days after the beginning of arthropod sampling. Later, two other releases were made every 20 days, totaling three releases during the experimental period. The chrysopid larvae were released in the ratio of two larvae per plant, totaling 400 chrysopid larvae per release. The larvae were released between eight and nine a.m., in the middle part of randomly chosen rosebushes in the beds.

In conventional rosebushes cultivation, the management and cultural practices normally used by the farmer were adopted, that is, phytosanitary treatments with chemical products (insecticides, acaricides, and fungicides) at a fixed schedule and without pest monitoring, applied once a week, with mixtures of up to three products. The insecticides and/or acaricides used were: abamectin (Abamectin Nortox®; 1.69 Oz 100 L^{-1}), propargite (Omite 720 EC®; 1.01 Oz 100 L^{-1}), fenpropathrin (Danimen 300 EC®; 0.84 Oz 100 L^{-1}), chlorfenapyr (Pirate®; 1.69 Oz 100 L^{-1}), pyriproxyfen (Cordial 100®; 2.7 Oz 100 L^{-1}), dimethoate (Dimexion®; 2.7 Oz L^{-1}), acetamiprid + pyriproxyfen (Privilege®; 100 g 100 L^{-1}) and mineral oil (Agefix®; 1 L 100 L^{-1}). The fungicides were: chlorothalonil (Daconil BR®; 0.44 lb 100 L^{-1}), tebuconazole (Tebuconazole CCAB 200 EC®; 2.53 Oz 100 L^{-1}), thiophanate-methyl (Cercobin 700WG®; 0.15 lb 100 L^{-1}), boscalid + kresoxim-methyl (Collis®; 1.69 Oz 100 L^{-1}), mancozeb (Alicerce®; 0.44 lb 100 L^{-1}), pyraclostrobin (Comet®; 1.35 Oz 100 L^{-1}) and mancozeb + metalaxyl-M (Ridomil Gold MZ®; 0.22 Oz 100 L^{-1}).

Weekly samplings of arthropods present on both the rosebushes in each treatment and on the basil were conducted. Evaluations started seven days prior to the first release of *C. externa*, totaling 10 evaluations. Samplings on the rosebushes were based on the number of arthropods present on three leaves per plant, which were randomly taken in the upper, middle, and lower thirds of each sampled plant [16]. Twelve rosebushes were randomly sampled per flowerbed, totaling 48 plants evaluated per treatment. Sampling of basil was done by beating the aerial part of the plant on a white plastic tray to dislodge the arthropods present, which were suctioned using a manual aspirator. All sampled arthropods were subsequently transported to the laboratory for counting and identification. Taxonomic classification of arthropods was carried out with the aid of specific dichotomous identification keys. Arthropods were classified into phytophages, predators, parasitoids, and pollinators. The yield and quality of flowers produced in each treatment (diversified rosebush or conventional rosebush) were also evaluated by counting the number of stems harvested at the commercial harvest point in each treatment. The commercial harvest point comprised the phase in which the petals of the extremity of the floral bud were curled together forming a well-defined spiral. Thus, stems were considered commercially produced when they were straight, without twisted flower buds or any other formation defects and with lengths of 15.75, 19.68 and 23.6 in [17].

Data concerning the sampling of all arthropods captured in the diversified rosebush, conventional rosebush, and basil plants were subjected to homogeneity of variance analysis and compared by the Kruskall-Wallis test ($p<0.05$). Graphical analyses of the arthropods found in the diversified rosebush and conventional rosebush were conducted. The ecological parameters of abundance (N); Shannon's diversity index (H') and Simpson's dominance index (D) were estimated. Pearson's linear correlation analysis ($p<0.05$) was conducted to evaluate the relationship of aphid abundance with the population of the natural enemies sampled in the diversified rosebushes. All tests were conducted using PAST® Software version 4.05 [18].

III. RESULTS AND DISCUSSION

A total of 3,122 arthropod specimens were sampled in the diversified rosebush, the conventional rosebush as well as in the basil plants. The collected arthropods belonged to 13 different taxa (Table 1). A greater abundance of specimens was observed in the diversified rosebush (2,222 specimens) compared to the conventional rosebush (423 specimens) and basil (477 specimens) (Table 1).

TABLE 1
TAXONS, ABUNDANCE, RELATIVE PERCENTAGE RECORDED IN THE DIVERSIFIED ROSEBUSH (rosebush + basil + *Chrysoperla externa* release), CONVENTIONAL ROSEBUSH AND BASIL

Taxa	Conventional		Diversified		Basil	
	Total	%	Total	%	Total	%
Aeolothripidae (P)	0	0	0	0	35	7.3
Aleyrodidae (F)	90	21.27	10	0.45	0	0
Anthocoridae (P)	0	0	0	0	29	6.07
Aphididae (F)	43	10.18	1175	52.9	0	0
Apidae (Po)	0	0	0	0	238	49.9
Araneae (P)	0	0	16	0.72	90	18.86
Braconidae (Pa)	0	0	74	3.33	0	0
Chrysopidae (R)	0	0	5	0.22	4	0.83
Coccinellidae (Po)	0	0	724	32.58	11	2.3
Ichneumonidae (Pa)	0	0	0	0	46	9.7
Miridae (P)	0	0	27	1.21	0	0
Syrphidae (P)	15	3.54	55	2.47	24	5.04
Thripidae (F)	275	65.01	136	6.12	0	0
Abundance (N)	423	100	2222	100	477	100
Shannon (H')	0.96		1.205		1.526	
Simpson (D)	0.5205		0.6085		0.6939	

***Ecological strategies F= Phytophagous, P= Predators, Pa= Parasitoids, Po= Pollinators**

The main phytophagous arthropods that were found on the rosebush belonged to the families Aphididae (aphids), Thripidae (thrips) and Aleyrodidae (whiteflies). The aphids species collected were *Aphis gossypii, Macrosiphum euphorbiae, Macrosiphum rosae, Myzus persicae and Rhodobium porosum* (Hemiptera: Aphididae). The thrips species collected were *Frankliniella occidentalis, Frankliniella shultzei, Thrips tabaci* and *Caliothrips phaseoli* (Thysanoptera: Thripidae). The whitefly species sampled was *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae). These phytophagous species are considered important pests in rosebush cultivation [2,3,10,13,16].

In the diversified rosebush area, the aphids were the most abundant phytophagous arthropods with 1,175 specimens collected (Table 1, Fig. 1). No damage was observed on the rosebushes in this treatment due to aphid attack. This was possibly due to the biological control provided by the chrysopid releases in the area, as well as the natural control provided by the natural enemies present on this side of the greenhouse (Table 1, Fig. 1). Several insects can be kept at densities below damage levels by natural enemies that naturally occur in the crop, such as predators and parasitoids that invade the greenhouse and result in a natural control of the pest [2,3].

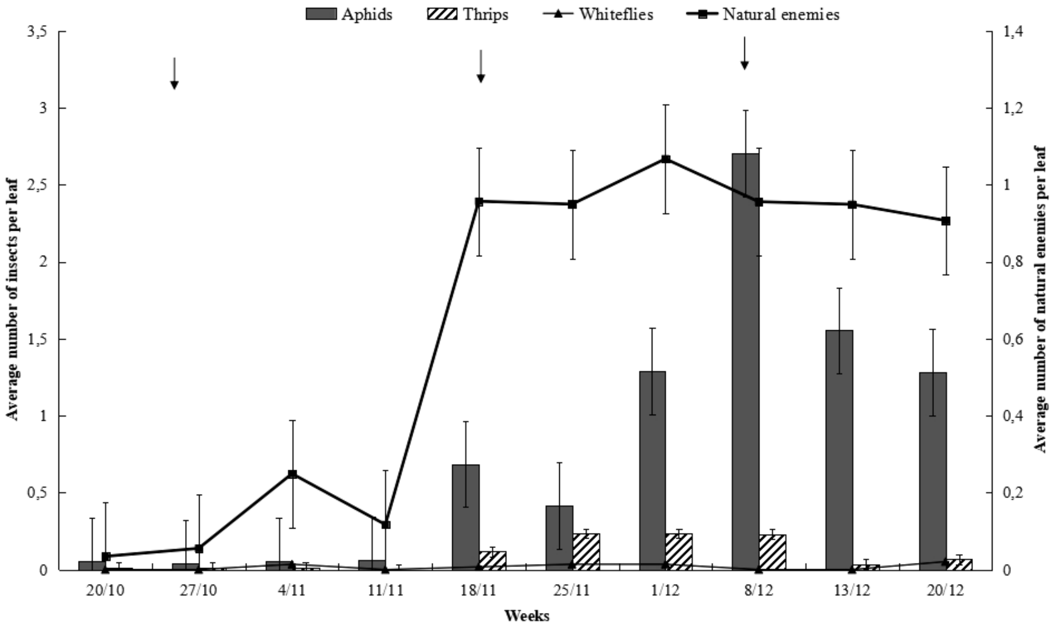

FIGURE 1: Average number of aphids, thrips, whiteflies and natural enemies per leaf of diversified rosebush (rosebush + basil + *Chrysoperla externa* releases) in the greenhouse. *Vertical arrows show the three releases of *Chrysoperla externa* larvae.

Among the natural enemies sampled in the rosebush and basil, we verified the presence of predators such as chrysopids (*C. externa*), ladybugs *Cycloneda sanguinea, Hippodamia convergens, Eriopis connexa, Psyllobora* sp. and *Scymnus* sp. (Coccinellidae), pirate bugs *Orius insidiosus* (Anthocoridae), predatory mirids *Hyaliodes beckeri* (Miridae), predatory thrips *Franklinothrips vespiformis* (Aeolothripidae), syrphids *Allograpta exotica, Pseudodorus clavatus* and *Toxomerus* sp. (Syrphidae) and spiders (Araneae). The parasitoids sampled were *Praon volucre* (Braconidae) and *Pimpla croceiventris* (Ichneumonidae). Most of these species have a polyphagous feeding habit and may be responsible for population control of different herbivore-pests in the agroecosystem [7,8,9,10,13,14,16].

In the diversified rosebush (rosebush + basil + *C. externa* releases), it was observed higher abundance of natural enemies (H: 13.17; p= 0.0001423), predators such as ladybugs (Coccinellidae), green lacewings (Chrysopidae), mirids (Miridae), syrphids (Syrphidae), spiders (Araneae) and parasitoids (Braconidae), having a total of 901 specimens collected, compared to the conventional rosebush (Fig. 2).

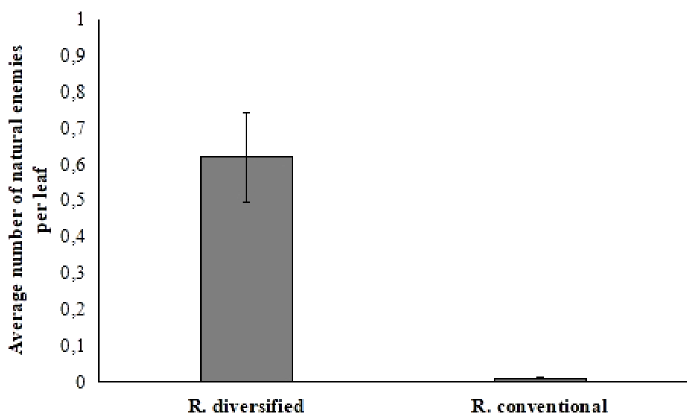

FIGURE 2: Average number of natural enemies per leaf in diversified rosebush (rosebush + basil + *Chrysoperla externa* releases) and in conventional rosebush in greenhouse. Treatments differed from each other by Kruskal-Wallis test at 5% probability.

The natural enemies found in this study may have contributed to the reduction of the population of phytophagous arthropods in the diversified rosebush cultivation. A significant and positive correlation, calculated by Pearson's coefficient, was found between the number of aphids and natural enemies present per rosebush leaves in the diversified area (r = 0.9229; P = 0.00014075) (Fig. 3). These results showed that natural enemies (ladybugs, green lacewings, pirate bugs, mirid bugs, syrphids, predatory thrips, spiders, and parasitoids) had significant and high correlations when not exposed to chemical insecticides, showing that these control agents can respond numerically to increased aphid infestation on rosebush and control their populations. This dependence between these insect groups may be associated with ecological relationships among them.

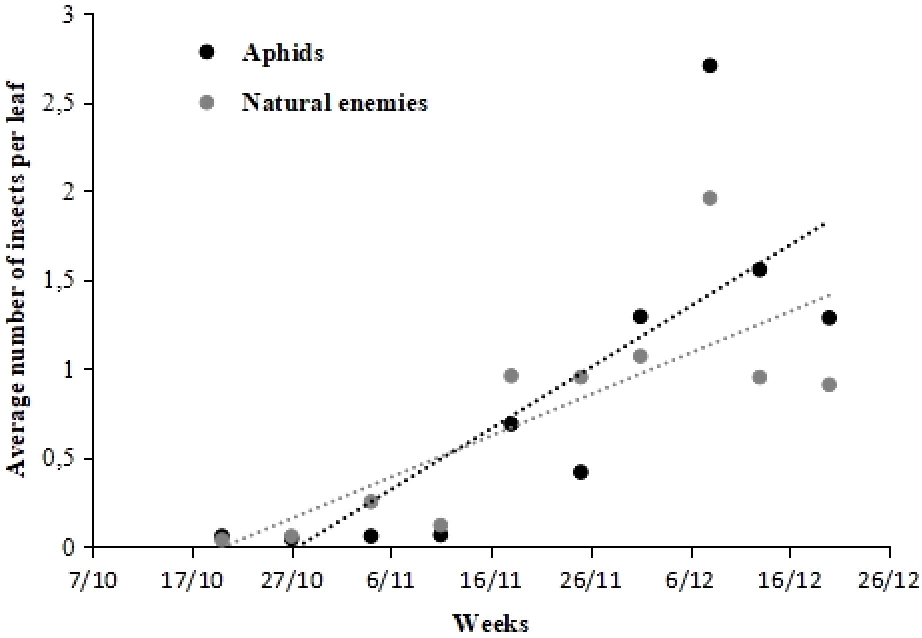

FIGURE 3: Average number of aphids and natural enemies per leaf of diversified rosebush (rosebush + basil + *Chrysoperla externa* releases) in greenhouse.

Constant and increasing presence of natural enemies was observed in the diversified rosebush cultivation area (Fig. 1), and this fact may be related to the use of basil as an attractive plant on the sides of the greenhouse. The fact that the basil plant had a constant bloom during the whole experimental period may have contributed to the attraction of a greater diversity of natural enemies to the area of diversified roses. This fact has also been confirmed when the diversity of species in the different treatments was evaluated, because it was verified a relatively high diversity by both Shannon-H index and Simpson index (H' = 1.205, D = 0.6085) in the diversified rosebushes, as in basil (H' = 1.526, D = 0.6939) (Table 1). These results demonstrated that the association of rosebushes and basil attract and host major natural enemies and that there is an increase in the abundance of these insects due to the availability of alternative food resources, mainly associated with basil flowers. According to [19] the long flowering period of basil, which lasts from three to four months, can benefit the combined crop by attracting beneficial insects and pollinators, since its flowers are considered a source of nectar with high sugar production.

Chrysopids assisted in reducing the aphid population in the diversified rosebush crop. After the releases of *C. externa* larvae there was a decrease in the occurrence of aphids on the rosebushes (Fig. 1). Studies show that *C. externa* larvae feed on aphids causing a decrease in their populations [10,13]. Despite the release of *C. externa* in the diversified rosebush, five specimens were found in the diversified rosebush and four in basil (Table 1). This difficulty in finding the chrysopids in the evaluations may have occurred due to the behavior of this predator, since this insect predator has a nocturnal habit, and the evaluations were made in the morning period. During the day, the adult chrysopids remain most of the time at rest on the lower surface of the leaves and the larvae remain inside the plants [20].

Adults of *C. externa* are not predators and mainly feed on sugary substances such as honeydew, floral and extrafloral nectar, and pollen [7,8,10]. These alternative food sources may be found in abundance in basil, which may be an important food source for this and other predators. Pollen from various botanical families were observed in the digestive tract of *C. externa* showing the importance of plant diversification in the growing area [7].

Among the predators sampled in the diversified rosebush the ladybugs were the most abundant with 724 specimens. This may have occurred because of the presence of prey in the diversified area and also because of the presence of basil that was constantly in bloom thus favoring the permanence of this predator in the area. Our results are consistent with [5] who reported that ladybugs apart from feeding on prey, also need floral resources such as pollen and nectar, which are able to ensure the survival of adults and sustain the metabolism and development of the young phase of this predator and the results of [9] when studying the influence of the association of kale with flowering plants, also found significantly higher numbers of individuals and species of these predatory insects in diversified cultivation, when compared to isolated cultivation.

In the samplings conducted on basil plants, a total of 477 specimens were collected, with pollinating insects (bees) being the most abundant with 238 specimens collected, among them *Apis mellifera* (Apidae) and other individuals of the Apidae family (Table 1; Fig. 2). Floral resources of basil provided higher pollinator richness and abundance, mainly *A. mellifera*, in sweet pepper crop associated with basil, compared to sweet pepper alone [19].

The spider group was the second most abundant taxon in basil, with 90 specimens collected, and their presence was observed in almost all collections. Spiders are predators of aphids and other arthropods and may be observed in various types of crops, as well as being bioindicators of environmental quality [21].

In basil, syrphids, thrips predators, parasitoids, spiders, pirate bugs, ladybugs and green lacewings were also sampled (Table 1). The constant presence of different beneficial insects that are attracted to the basil plant may have helped to reduce the aphid population and other phytophages in the diversified roses.

In order to control pest populations in commercial crops, the natural enemies must be nearby the plants prior to the outbreak of pests and this can only be achieved by first attracting these biological agents and providing conditions for their establishment in the area [7,8,10], since the population of natural enemies depends on the pest population, if there is no availability of other food resources the efficiency of biological control is impaired, because of the rapid increase of arthropod-pests. The use of basil combined with sweet pepper cultivation made it easier to install *Orius laevigatus* (Hemiptera: Anthocoridae), an important predator of thrips, aphids, whiteflies and mites [14]. Basil flowers have provided higher survival for larvae and adults of *Ceraechrysa cubana* (Neuroptera: Chrysopidae) and the use of *O. basilicum* as a diversification component in agricultural areas may be of benefit in attracting and maintaining *C. cubana* populations favoring biological control [11].

The diversified rosebush crop was the closest to the predator species found on basil. They shared 5 taxa, this may be beneficial to the rosebush crop because of the high number of predators and parasitoids found in the area. The Aeolothripidae, Apidae, Anthocoridae taxa were unique to basil and the Miridae taxon was unique to the diversified cultivation, being classified as predators or pollinators. This fact shows the importance of maintaining basil plants near the rosebushes in the greenhouse, since through the results found, it was observed that basil plants can serve as a reservoir for these predators. Authors have reported that in periods of scarcity of preys, natural enemies can use pollen and nectar from flowering plants nearby the cultivation area for feeding, as well as use these flowering plants as shelter and/or oviposition sites [7,8,11,14].

The higher abundance of predatory insects observed during the experiment in the diversified rosebush is in line with the "Natural Enemy Hypothesis" [22], where it states that biological control agents tend to be more abundant in diversified environments, because these provide shelter, sites for reproduction, which promotes the establishment and multiplication of these insects.

In conventional rosebushes, despite the repeated weekly spraying of chemical products, the occurrence of several phytophagous insects was observed, especially thrips, with 275 specimens sampled (Fig. 4). Furthermore, practically no natural enemies were observed during the sampling in the conventional rosebush (Fig. 4). This may have occurred because of the intensive use of non-selective chemicals (insecticides, acaricides, and fungicides) in this area. Several authors have reported that the use of chemicals is quite common in the cultivation of rosebushes, negatively influencing the populations of natural enemies, causing resistance problems and hindering the biological control of pests [1,2,3,4].

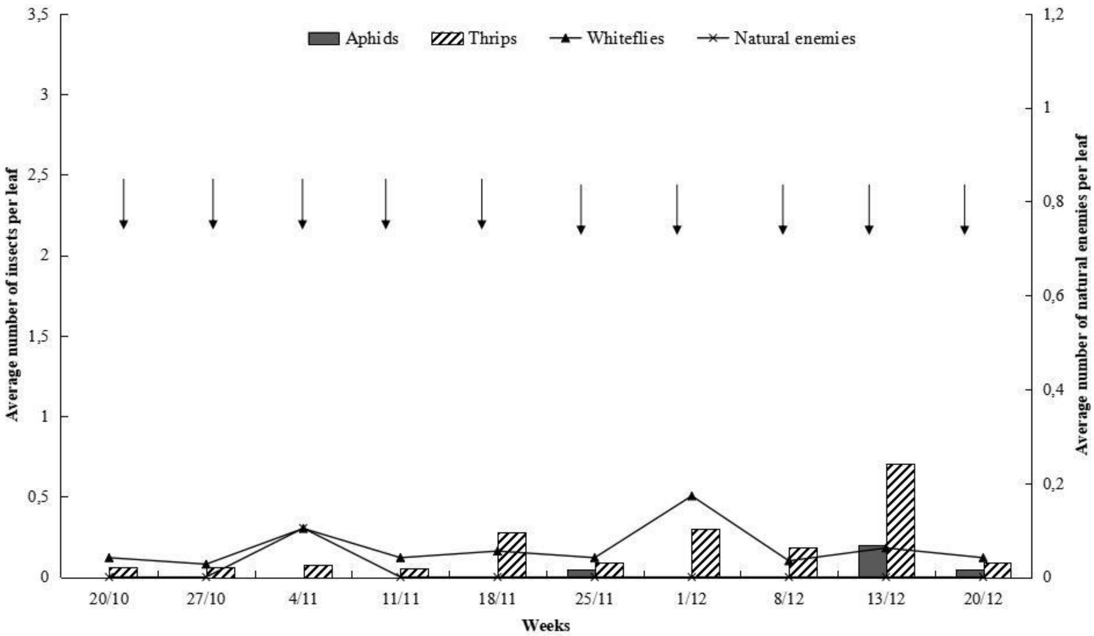

FIGURE 4: Average number of aphids, thrips, white flies and natural enemies in conventional rosebushes (sprays with insecticides, acaricides and fungicides) in a greenhouse. *Vertical arrows indicate weekly application of chemicals (insecticides, acaricides and fungicides).

As regards the productivity of rose floral stems, it was found that the number of stems harvested was not affected by the type of treatment evaluated, that is, an average of 57.9 stems were harvested in the diversified rosebush cultivation and 59.7 stems in the conventional rosebush cultivation. Moreover, it was verified that all harvested stems had the commercial quality standard, according to [17].

In view of the results obtained, it was possible to verify that the implementation of sustainable agroecological pest management practices in rosebushes, such as the combination of using basil as an attractive plant and chrysopid releases, favored biological control in the cultivation of rosebushes and did not affect the production and quality of the rose stems produced. In addition, no chemicals were applied in the agroecological rosebushes, thus avoiding problems of environmental contamination and residues in the flowers. However, further studies should be carried out in order to better understand the effect of basil on the conservative biological control of roses, because the knowledge of these ecological relationships may be important for bioecology studies and also as a tool for planning and implementing pest management in rosebushes.

IV. CONCLUSION

The combination of using basil as an attractive plant and chrysopid releases favors the biological control in the cultivation of rosebushes in a greenhouse. Basil associated with rosebushes provides greater abundance and diversity of natural enemies in rosebushes. Basil is a promising attractive plant for conservation biological control in roses. The production and quality of floral stems are not impaired by diversification of rosebushes with basil.

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