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Predators of the Alfalfa Aphids *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch, and *Therioaphis trifolii* (Monell) (Hemiptera: Aphidoidea) as Determined by the Serological Technique

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Abstract. The serology is an immunological technique based on antigen/antibody reactions in where its main advantages are high sensitivity and specificity that allows the biological recognition at the molecular level. This study evaluates the use of serology technique to determine the predators of the alfalfa aphids *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch, and *Therioaphis trifolii* (Monell) (Hemiptera: Aphidoidea). The aphid samplings to obtain the antibodies and their possible predators to be used as antigens were carried out in the alfalfa fields of the Embrapa Pecuária Sudeste Unit, São Carlos, SP. In the period from August 2011 to July 2012. A total of 2,161 arthropod predators, including insects and spiders, were tested. The antibodies obtained for the aphid *A. craccivora*, *A. pisum*, and *T. trifolii* showed partial identity nevertheless still allowed to recognize the predators of alfalfa aphids. Among the insects, syrphids and chrysopids presented the highest percentage of positive results in the serological tests. The species *A. craccivora* was the most preyed aphid.

Keywords: Antibodies; biological control; predation; serology.

Determinação de Predadores dos Pulgões da Alfafa *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch e *Therioaphis trifolii* (Monell) (Hemiptera: Aphidoidea) por meio da Técnica Serológica

Resumo. A serologia é uma técnica imunológica baseada em reações antígeno/anticorpo, em que suas principais vantagens são a alta sensibilidade e especificidade que permitem o reconhecimento biológico em nível molecular. Este trabalho avalia o uso da técnica serológica para determinar os predadores dos pulgões da alfafa, *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch e *Therioaphis trifolii* (Monell) (Hemiptera: Aphidoidea). As coletas dos pulgões para a obtenção dos anticorpos e de seus possíveis predadores para serem utilizados como antígenos foram realizadas nos campos de alfafa da Unidade da Embrapa Pecuária Sudeste, São Carlos, SP. no período de agosto de 2011 a julho de 2012. Foram testados 2.161 artrópodes predadores, incluindo insetos e aranhas. Os anticorpos obtidos para os pulgões *A. craccivora*, *A. pisum*, e *T. trifolii* mostraram identidade parcial mas, ainda assim, permitiu reconhecer os predadores dos pulgões da alfafa. Dentre os insetos, sirfídeos e crisopídeos foram os que apresentaram as maiores porcentagens de resultados positivos nos testes serológicos. *A. craccivora* foi o afídeo mais consumido pelos predadores.

Palavras-chave: Anticorpos; controle biológico; predação; serologia.

Alfalfa, *Medicago sativa* L., is one of the most cultivated forage plants worldwide, being the United States, Russia, Canada, and Argentina the main producers. The area cultivated with alfalfa in Brazil is around 30,000 ha, which is concentrated in the southern region and is responsible for 90% of total Brazilian production (Pereira 2008). The aphids *Acyrthosiphon pisum* Harris (Aphididae: Macrosiphini), *Aphis craccivora* Koch (Aphididae: Aphidini), and *Therioaphis trifolii* (Monell) (Drepanosiphidae: Phyllaphidini) are current pests of alfalfa. They damage the alfalfa plants by shortening the plant internodes, wrinkling the leaves, thus hindering the plant growth (Afonso 2008).

Outbreaks of alfalfa aphids are controlled by using chemical insecticides, which are dangerous not only to the environment, but also to alfalfa-feeding animals. Biological control is a good alternative strategy to the use of chemicals; therefore it is very important to have knowledge on the occurrence of predators' species of the alfalfa aphids. Among these predators adults and immature Geocoridae, Nabidae, Anthocoridae (Hemiptera: Heteroptera), Coccinellidae (Coleoptera) Chrysopidae (Neuroptera) and Syrphidae (Diptera) larvae are recognized as important aphid predators in alfalfa (Bueno & Silva 2008).

Direct observation is theoretically the simplest method to determine the predatory actions, however, hardly applicable to small animals, such as arthropods, or those that are difficult to approach (Dajoz 1973).

Immunoassay techniques like Serology and Polymerase Chain Reaction (PCR) show advantage over direct observation by minimizing researcher interference in the environment and by not ruling out the occurrence of nocturnal predators. Serology is cheaper than PCR because it does not require sophisticated equipment and provides stable results and unlimited content of antibodies, allowing investigation of a large number of natural enemies (Hardwood & Obrycki 2005).

The serological technique is based on antigen / antibody reactions where the antibodies are obtained using the investigated aphids and the antigens obtained from the various aphid consumers.

This study aimed to produce antisera from the aphids *A. pisum, A. craccivora* and *T. trifolii* and use them to determine their predators in alfalfa using the serology technique.

MATERIALS AND METHODS

The aphids *A. pisum, A. craccivora*, and *T. trifolii*, as well as the group of predators used in this research were collected from alfalfa plants (cv. Crioula) in experimental fields of Embrapa Pecuária Sudeste-CPPSE, in São Carlos, state of São Paulo, Brazil, from August 2011 to July 2012. The aphids *A. pisum, A. craccivora*, and *T. trifolii* were used to obtain the antisera AsAp, AsAc, and AsTt, respectively, and the predators were used as antigens in the serological reactions.

Samples of each aphid species were separated and kept for 24 h. Thereafter, the samples were macerated in a 0.85% saline solution and centrifuged for five min. at 6,000 *g*. The precipitate was discarded, and 0.5 mL of the supernatant emulsified with Freund's incomplete adjuvant at 1:1 a ratio (v/v). The emulsified macerate was inoculated in a rabbit at 10-day intervals. We performed 10 inoculations with *A. pisum* (AgAp) macerate, 19 inoculation with *A. croccivora* (AgAc) macerate, and 11 inoculations with *T. trifolii* (AgTt) macerate.

During the inoculation period, we performed weekly bloodsheds in each animal for antisera acquisition and evaluation of their respective titers. The blood samples (10 mL on average) were obtained from small longitudinal incisions on the marginal veins of the rabbit's ears. Each sample remained for 2 h at room temperature; the coagula were discarded and the resulting antisera were stored at -2 °C for later use in serological reactions. These reactions were performed by double diffusion in 1% agar gel (Ouchterlony 1958) with PBS buffer, pH 7.0, on slides (3 mL agar solution per 75 x 25 mm slide). The titers were performed by using fractions with 0.85 % NaCl solution of each AsAp, AsAc, and AsTt antisera in reactions with their respective homologous antigens AgAp, AgAc, and AgTt. The dilutions were performed according to a geometrical progression ratio 2.

RESULTS

The first positive results between each antiserum and its homologous antigen were observed seven days after the first inoculation. The titers obtained from each antiserum were 1/32 for AsAp, 1/32 for AsAc, and 1/8 for AsTt, after the 3rd, 4th, and 5th inoculation in each rabbit, respectively. In addition, homologous reactions with each antiserum also reacted with the different alfalfa aphids, showing a partial identity. We collected 2,161 specimens of predaceous insects and spiders: among the insects, specimens of Coleoptera (Carabidae and Coccinellidae), Dermaptera, Diptera (Dolichopodidae and Syrphidae), Hemiptera (Geocoridae, Nabidae, Pentatomidae, and Reduviidae), and Neuroptera (Chrysopidae) were recorded; whereas for the spiders, specimens of the families Araneidae, Ctenidae, Lycosidae, Linyphiidae, Nephilidae, Pholcidae, Salticidae Sicariidae, Sparassidae, Theridiidae, Thomisidae, and Trochanteriidae were identified.

From the 508 predators tested by the serological technique, 23.5% reacted with at least one aphid antiserum. The syrphids showed the highest percentage of positive results (67.2%). The chrysopids accounted for 65% of positive results, making evident their importance as aphid predators in the alfalfa crop. Larvae of Coccinellidae were responsible for 47.8% of the positive results, while the adults were responsible for 22%; *Hippodamia convergens* Guérin-Méneville stood out in this group with 30.5% of positive results. Regarding the Hemiptera, the reduviids represented 41.1% of positive results, followed by the nabids (33.9%), geocorids (27.2%), pentatomids (5.2 %), and lastly Dermaptera (7%).

Concerning the spiders (Araneae), the family Pholcidae highlighted with 16.6% of positive results, and was followed by Nephilidae (14.28%), Araneidae (9.0%), Lycosidae (7.69%), Salticidae (7.14%), Thomisidae (6.75%), and Ctenidae (5.28%).

The serological reactions between each antisera and the different predators showed 392 positive results with the antiserum for *A. cracivora*, 296 with the antiserum for *A. pisum*, and 224 with the antiserum for *T. trifolii* (Table 1).

We observed that the sum of positive results with each antiserum is not always equal to the total number of positive results, since the same predator may have preyed on one, two or three aphid species. Moreover, the partial identity among the antisera may lead to false positive results. We observed a higher number of positive reactions with AsAc, that is, with predators that probably consumed *A. craccivora*, even this aphid being the less abundant species during the collecting period (Table 2).

Table 1. Results obtained from serological reactions among predators collected in alfalfa and the antibodies AsAc (*A. craccivora*), AsAp (*A. pisum*), and AsTt (*T. trifolii*).

Predator	Number of tested predators	Number of positive results	Results with each antiserum		
			AsAc	AsAp	AsTt
Coccinellidae (adults)	1402	309	231	178	108
Hippodamia convergens Guérin-Méneville	409	136	74	81	28
Harmonia axyridis (Pallas)	748	127	84	61	46
Cycloneda sanguinea (L.)	213	38	65	31	29
Eriopis connexa (Germar)	32	8	8	5	5
Coccinellidae (larvae)	71	34	27	13	27
Chrysopidae	20	13	11	10	4
Syrphidae	55	37	36	29	23
Reduviidae	17	7	7	3	4
Carabidae	24	3	3	0	1
Pentatomidae	76	4	3	2	3
Geocoridae	55	15	11	8	9
Dermaptera	13	1	1	0	0
Nabidae	156	53	41	38	24
Araneae	272	32	21	15	21
Total	**2161**	**508**	**392**	**296**	**224**

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Table 2. Number of aphids sampled for each species in alfalfa from August 2011 to July 2012.

Period	*A. pisum*	*A. craccivora*	*T. trifolii*
Spring	491	430	1658
Summer	3734	1377	2056
Autumn	1228	227	566
Winter	314	535	494

DISCUSSION

Among the tested predators, syrphids stood out for presenting the highest frequency of positive results. Syrphids are considered to be essential predators of aphids in Portugal (Ilharco 1992), as well as potential predators of many aphid species with economic importance foragricultural crops in Brazil (Bastos & Torres 2005). Chrysopids also highlighted for the high percentage of positive results, being surpassed only by the syrphids. According to Bastos & Torres (2005), although being able to prey on a diverse array of insect species, chrysopids prey preferably on aphids; during their larval stage chrysopids can consume from 100 to 600 aphids. The coccinellids stand out as aphid predators (Saran *et al.* 2007) and, even without any species of this exclusively afidophagous family, some have strong preferences for these insects, such as *Cycloneda, Harmonia* and *Hippodamia* (Giorgi *et al.* 2009), as observed in the present study.

Kato *et al.* (1999) reported that *H. convergens* is responsible for maintaining alfalfa aphid populations below the threshold levels in California. Saran *et al.* (2007) stated that larvae of some coccinellid species have a daily prey intake higher than that showed by the adults. Daily consumption of *Cycloneda sanguinea* (L.) larva, for instance, may reach 200 aphids, while the adult consumes only 20 aphids per day on average.

Spiders are generalist predators. Besides using the food that is trapped in their webs they are also avid hunters (Saran *et al.* 2007). In the present study spiders were observed both in the soil and in the higher parts of the alfalfa plant. Greenstone & Hunt (1993) stated that serology is an important method to studying preying by spiders; because only the prey's body fluids are consumed by spiders, it is difficult to identify the prey using other techniques. Sunderland *et al.* (1987) mentioned Lycosidae and Tetragnatidae as important families of aphid predaceous spiders in cereal crops. Although Ilharco (1992) reported some evidence that spiders might prey on aphids, little interest has been shown to this group as biological control agents of aphids.

According to Sunderland *et al.* (1987), the high variation in aphid detection by using the serological technique may be attributed to the prey size, and even to the predator size. The aphids *A. craccivora* and *T. trifolii* have similar body sizes, varying from 1.4 to 2.2 mm lenght. Such slight variation in body size would not be the only factor responsible for prey choice by the predators. *A. pisum* body size ranges from 2.5 to 4.4 mm, and in spite of the higher body size, this aphid was not the least preyed species among the tested aphids. These results indicate that besides the body size, other factors, such as prey palatability and toxicity, may influence predator searching and choice for most suitable preys.

The serology, being a highly sensitive and specific technique can be successfully used to qualify predators in studies of food relationships, especially taking into account small organisms such as arthropods. In biological control programs it allows to estimate the preference of a given predator for a certain prey.

In conclusion, the antisera obtained for the aphids *A. craccivora, A. pisum*, and *T. trifolii* showed partial identity. It was possible to recognize the alfalfa aphid predators by using the serology technique. Syrphids and chrysopids were the predators presenting the highest frequency of positive serological results. Highest frequencies of positive serological reactions occurred with the antiserum obtained for *A. craccivora* (AsAc).

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