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Egg parasitism of *Piezodorus guildinii* and *Nezara viridula* (Hemiptera: Pentatomidae) in soybean, alfalfa and red clover

Parasitismo de huevos de *Piezodorus guildinii* y *Nezara viridula* (Hemiptera: Pentatomidae) en soja, alfalfa y trébol rojo

María Fernanda Cingolani, Nancy Mabel Greco, Gerardo Gustavo Liljesthröm

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ABSTRACT

Piezodorus guildinii Westwood and *Nezara viridula* (L.) (Hemiptera: Pentatomidae) are important soybean pests. *P. guildinii* causes more injury and is less susceptible to insecticides compared to *N. viridula*. *N. viridula* egg parasitoids are well studied; however, little is known about parasitoids of *P. guildinii*. Alfalfa, soybean and red clover were sampled during several seasons to characterize the abundance of both stink bugs, to determine their egg parasitoids, and to estimate parasitoids impact. In the field, *Telenomus podisi* (Ashmead), *Trissolcus urichi* (Crawford) and *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) emerged from *P. guildinii*, while only *T. basalis* (Wollaston) (Hymenoptera: Platygastridae) emerged from *N. viridula*. The proportions of parasitized eggs (*i. e.*, the parasitoid impact) and egg masses, as well as the number of parasitized eggs/total number of eggs of the parasitized egg masses, were similar for alfalfa and soybean. Parasitism was not observed in red pclover. Parasitoid impact was lower during the dry growing seasons. Although *P. guildinii* field parasitism by *T. urichi* was less significant, laboratory experiments from the bibliography indicate that this wasp species performs well on this host. *Trissolcus urichi* would be an important biological control agent against *P. guildinii*, principally when the stink bug is more abundant.

RESUMEN

Piezodorus guildinii Westwood y *Nezara viridula* (L.) (Hemiptera: Pentatomidae) son importantes plagas en soja. *P. guildinii* causa mayores daños y es menos susceptible a los insecticidas. Los parasitoides oófagos de *N. viridula* fueron ampliamente estudiados, pero poco se conoce sobre los de *P. guildinii*. Se muestreó alfalfa, soja y trébol rojo durante varias temporadas para caracterizar la abundancia de ambas chinches, identificar sus parasitoides y estimar el impacto de los mismos. *Telenomus podisi* (Ashmead), *Trissolcus urichi* (Crawford) y *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) emergieron de *P. guildinii*, mientras que solo *T. basalis* (Wollaston) (Hymenoptera: Platygastridae) emergió de *N. viridula*. Las proporciones de huevos (impacto del parasitoide) y de posturas parasitadas, y el número de huevos parasitados/número total de huevos de las posturas parasitadas, fueron similares para alfalfa y soja. En trébol rojo no se registró parasitismo. El impacto del parasitoide fue menor durante las temporadas secas. Si bien el parasitismo de *P. guildinii* por *T. urichi* a campo fue poco significativo, los antecedentes de la bibliografía indican que su desempeño en el laboratorio sobre este hospedador es muy bueno. *T. urichi* podría ser un potencial agente de control biológico de *P. guildinii*, principalmente en situaciones de mayor abundancia de la chinche.

CEPAVE (Centro de Estudios Parasitológicos y de Vectores), CCT La Plata CONICET-UNLP. Calle 2 # 584, La Plata (B1902CHX). Buenos Aires, Argentina. Tel.: +54 221 4233471 (ext 32). Fax: +54 221 4232327. fernandacingolani@cepave.edu.ar; fernandacingolani@hotmail.com

Keywords

Telenomus podisi • *Trissolcus urichi* • *Trissolcus basalis* • field parasitism • performance

Palabras clave

Telenomus podisi • *Trissolcus urichi* • *Trissolcus basalis* • parasitismo a campo • desempeño

INTRODUCTION

Habitat heterogeneity is a general feature of considerable importance for many species, and human fragmentation of the landscape is likely to have considerable influence in community and population dynamics (19, 33).

The great expansion of soybean crop in recent years in Argentina has caused significant changes, particularly in the Rolling Pampas region. One such change is the supply of soybean as a food resource for some phytophagous species, like stink bugs of the family Pentatomidae, on larger areas and for longer periods of time (1). It has been found that host plant species other than soybeans favor the persistence of pentatomids during critical periods of the year, and in some cases also allow population increase (20, 28). Before soybean is available in the field, the post-hibernating adult stink bugs reproduce a first generation on cultivated or feral legumes. In Argentina, the more important resources for post-hibernating pentatomids are alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) (Fabales: Fabaceae). From these hosts, they colonize soybean in the flowering stage or shortly before, and develop three relatively discrete generations (3). The quality of the habitats fragments, together with weather conditions, influence the development and survival of stink bugs invading soybeans. Moreover, these factors can also influence the performance of the natural enemies, which in turn affects the subsequent growth and population dynamics of the pest in the crop (12). Both in the soybean and in adjacent habitats, egg parasitoids are important natural enemies that limit the numerical increase of stink bugs.

Pentatomids are the most important seed sucking pests on soybean. They feed by inserting their stylets removing plant nutrients from either vegetative tissues or fruiting structures causing abortion of fruit or seed, and, in some instances vectoring diseases (29). In Argentina, the most common pests are the redbanded stink bug, *Piezodorus guildinii* Westwood, and the southern green stink bug, *Nezara viridula* (L.) (13). Until a few years ago, *P. guildinii* was not considered to be a serious problem in soybeans in Argentina, as it was considered to be a less serious pest than *N. viridula*. However, its relative abundance has been increasing significantly in the provinces of Santa Fe and Buenos Aires. Nowadays, *P. guildinii* is the most important species attacking soybean crops in Buenos Aires (21) and one of the most important in Brazil (10) and Uruguay (6), together with *N. viridula* and *Euschistus heros* (F.) (Hemiptera: Pentatomidae). *P. guildinii* has also become a significant pest of soybean crops in Arkansas and other states in the Mid-South of the United States of America (2).

Concerning stink bugs natural enemies, in the Neotropical region the most common egg parasitoid species are *Telenomus podisi* (Ashmead), *Trissolcus urichi* (Crawford), *Trissolcus basalis* (Wollaston), *Trissolcus brochymenae* (Ashmead), *Trissolcus teretis* Johnson (Hymenoptera: Platygastridae), *Ooencyrtus submetallicus* (Howard), *Ooencyrtus* sp. (Ashmead) (Hymenoptera: Encyrtidae), *Neorileya* sp. (Ashmead) (Hymenoptera: Eurytomidae) and three undetermined species of the family Pteromalidae (7, 11, 23).

The presence of alternative hosts in the field is an important factor in maintaining parasitoid populations when the most suitable host is scarce.

Objectives

- To characterize the relative importance of *P. guildinii* and *N. viridula* in soybean, alfalfa and red clover.
- To determine egg parasitoid species attacking *P. guildinii* and *N. viridula* egg masses in soybean, alfalfa and red clover fields of the province of Buenos Aires.
- To estimate the overall egg parasitism.

MATERIALS AND METHODS

This study was conducted in alfalfa, red clover and soybean fields planted in the Experimental Station "Julio Hirschhorn" of the Faculty of Agricultural Sciences and Forestry of the National University of La Plata (34° 59' 24.32" S, 58° 0' 18.51" W; 27 m s. n. m.). Planting in the five years was conducted between 4th and 20th november. Alfalfa was kept permanently in the field. Plots were of about 3.500m2 each, and the distances between the three crops plots was around 150m, being the three crops approximately equidistant. Soybean, alfalfa and red clover were sampled weekly during five growing seasons (December through April) from 2006-2007 to 2010- 2011 for soybean, and over three growing seasons (from 2008-2009 to 2010- 2011) for alfalfa and red clover. Soybean was sampled from the beginning of flowering, in late December, to harvesting, in early April (approximately 14 weeks). Sampling in alfalfa and red clover were made from the vegetative state (mid-December) to dry pod state (early May) (approximately 19 weeks). Management practices conducted in the soybean field consisted only of the application of herbicide (glyphosate) 15 to 20 days before and after planting and an application of the fungicide carbendazim about a month after planting when soybean was still in the vegetative stage. Alfalfa and red clover fields were not chemically treated. Data of temperature and precipitation were provided by the Argentine National Weather Service for the study area (32).

We used the most suitable egg masses sampling method in crop. On soybean, each sample consisted of all the *P. guildinii* and *N. viridula* egg masses present in 400 randomly selected plants. On alfalfa and red clover, each sample consisted of all the *P. guildinii* and *N. viridula* egg masses collected in 10 sweep nets catched across

10 meters from each of 15 transects randomly arranged within the field. While we did not quantify the effect of egg masses removal on their density on the next sample, it is considered that the number of plants from which egg masses were obtained let us estimate parasitoids impact indicators without significantly affecting stink bugs populations. Whereas planting was conducted with 50cm between rows and about 14 plants per meter of row, the 400 plants from which we extracted egg masses represented less than 1% of the total plants in the plot. All egg masses found were collected and taken to the laboratory and kept individually in test tubes plugged with cotton at 20°C, 70%RH and 14h of photophase, until the emergence of nymphs and/ or wasps. The number of eggs per egg mass, the number of emerged nymphs, and the number, sex and species of emerged parasitoids were registered for each host.

Parasitoids emerged in the laboratory were determined to species using the keys of Johnson (17, 18), and species identifications verified (see Acknowledgements). Twenty days after collection, unhatched eggs were dissected to assess the presence and identity of parasitoids dead inside the host.

Egg masses that were found already emerged, *i. e.* with opened operculum (evidence of the emergence of nymphs) or with perforated operculum (evidence of the emergence of a parasitoid), were also considered to estimate the impact of parasitoids.

Parasitoids found dead inside host egg were identified to the species level when individuals were developed until the adult stage. In cases in which we could not identify individuals to the species level, they were considered only to estimate the impact of parasitoids, as done with egg masses that were found already emerged, with perforated operculum (see above). We estimated the overall impact of parasitism using the indices defined by 4: (1) the discovery efficiency (DE): number of parasitized egg masses/total number of collected egg masses; (2) the parasitism efficiency (PE): total number of parasitized eggs/total number of eggs of the parasitized egg masses; (3) the parasitoid impact (PI): total number of parasitized eggs/total number of collected eggs.

We compared each index among crops by one-way ANOVA using data of the growing seasons as replicates. Parasitism efficiency and parasitoid impact were also compared among growing seasons in a given crop using one-way ANOVA, taking each sampling date as a replicate. When ANOVA assumptions were not satisfied, the Kruskal-Wallis test was performed.

To evaluate if the number of parasitized egg masses in each crop was independent of the growing seasons, a chi-square analysis of contingency tables was performed.

RESULTS

Piezodorus guildinii was dominant in all three crops (94.30% of the collected egg masses) and *N. viridula* egg masses were collected only in soybean and its abundance was much lower (table 1, page 19).

Table 1. Total number of *P. guildinii* and *N. viridula* egg masses (and eggs) collected in each growing season, in soybean, alfalfa and red clover.

Tabla 1. Número total de posturas de *P. guildinii* and *N. viridula* (y huevos) colectados en cada temporada, en soja, alfalfa y trébol rojo.

crop	growing season	n° of egg masses (eggs)	
		P. guildinii	*N. viridula*
soybean	2006-2007	37 (676)	1 (84)
	2007-2008	30 (527)	3 (236)
	2008-2009	68 (1343)	4 (309)
	2009-2010	10 (199)	0
	2010-2011	51 (932)	12 (790)
alfalfa	2008-2009	19 (367)	0
	2009-2010	20 (415)	0
	2010-2011	75 (1272)	0
red clover	2008-2009	6 (75)	0
	2009-2010	7 (91)	0
	2010-2011	8 (98)	0
	TOTAL	331 (5995)	20 (1419)

The spatial and temporal abundance of *P. guildinii* egg masses was variable in the three studied crops (figure 1, page 20).

In alfalfa, the highest abundance of egg masses occurred between early February and mid March in the 2010-2011 growing season, coinciding with the end of the flowering and fruiting period of the crop.

In soybean, a first generation of low density was registered during January and the subsequent increase in the number of egg masses corresponded to the second generation in this crop, which peaked between late February and mid April in the different growing seasons. The greater abundance was coincident with the last part of the flowering period and with the whole fruiting period of the crop. A third generation was difficult to identify.

Piezodorus guildinii egg masses were parasitized by *T. podisi*, *T. urichi* and *T. basalis* in soybean and in alfalfa, while no parasitism was registered in red clover. *N. viridula* was parasitized only by *T. basalis*. The 76.22% of the wasps that emerged from field collected *P. guildinii* egg masses were individuals of *T. podisi*, denoting their predominance. Parasitism by this species was recorded during all the studied growing seasons, both in soybean and in alfalfa (table 2, page 21). In turn, *T. basalis* accounted for 6.77% of the wasps emerged. *T. urichi* was recorded only in 2010-2011 in both crops, accounting for 17.01% of the emerged parasitoids.

During this study, two different species of parasitoids emerged from an individual *P. guildinii* egg mass, in three occasions. In one case individuals of *T. basalis* emerged together with *T. podisi*, while in the other two cases the emergence of *T. podisi* and *T. urichi* was registered.

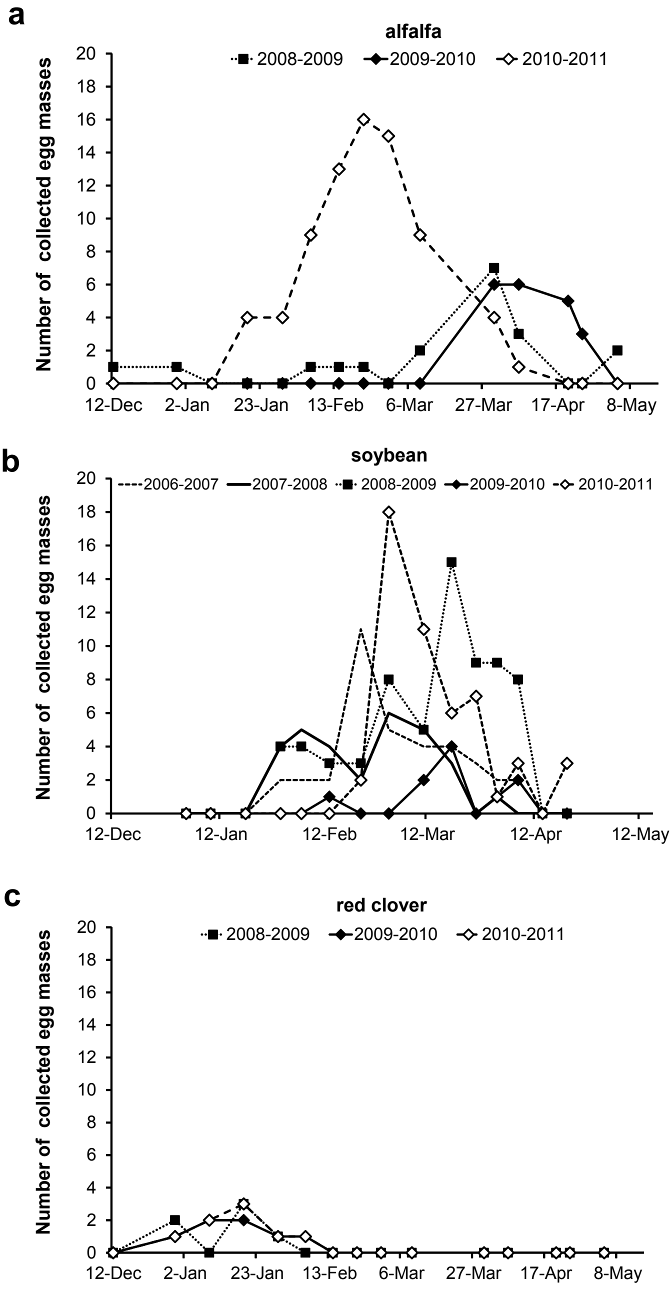


Figure 1. Number of collected *P. guildinii* egg masses in different crops and growing seasons. (a) alfalfa, (b) soybean and (c) red clover.

Figura 1. Número de posturas de *P. guildinii* colectadas en diferentes cultivos y temporadas. (a) alfalfa, (b) soja y (c) trébol rojo.

Table 2. Percent emergence for each parasitoid species, from parasitized eggs of *P. guildinii* and *N. viridula* in relation to seasons and crops (soybean and alfalfa).

Tabla 2. Porcentaje de emergencia de cada especie de parasitoide, a partir de huevos de *P. guildinii* y de *N. viridula*, en relación con las temporadas y los cultivos (soja y alfalfa).

Host	Parasitoid	soybean					alfalfa			red clover		
		2006 2007	2007 2008	2008 2009	2009 2010	2010 2011	2008 2009	2009 2010	2010 2011	2008 2009	2009 2010	2010 2011
	T. podisi	84.87%	100%	56.92%	100%	84.08%	60.71%	100%	57.56%	0	0	0
P. guildinii	*T. urichi*	0	0	0	0	7.27%	0	0	42.44%	0	0	0
	T. basalis	15.13%	0	43.08%	0	8.65%	39.29%	0	0	0	0	0
N. viridula	*T. basalis*	100%	100%	100%	0	100%	0	0	0	0	0	0

The overall discovery efficiency of *P. guildinii* egg parasitoids was not significantly different between crops (F = 0.05; df = 1, 6; P = 0.832), suggesting that the searching efficiency of the parasitoids would not be affected by particularities of the crop. On the other hand, the number of parasitized egg masses was affected by growing seasons both on soybean (χ^2 = 80.17; df = 4; P < 0.001) and in alfalfa (χ^2 = 9.00; df = 2; P = 0.011), and a lower number of parasitized egg masses was observed in 2007-2008 and 2008- 2009 relative to the other growing seasons (figure 2a, page 23). The overall discovery efficiency of *N. viridula* egg parasitoids was 0.65, and parasitism efficiency was very high (0.99).

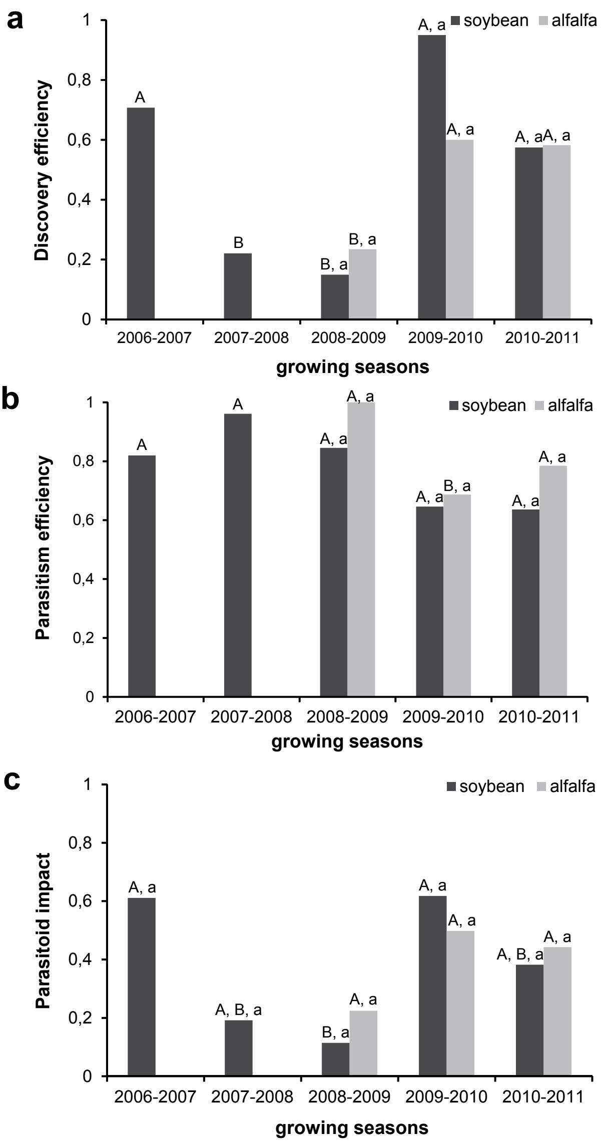
As above, parasitism efficiency of *P. guildinii* eggs was similar for soybean and alfalfa (figure 2b, page XXX) (F = 0.16; df = 1, 6; P = 0.707). However, this index was similar among growing seasons for soybean ($H_{(4,\ N = 30)}$ = 4.89; P = 0.300) while for alfalfa it was higher in 2008-2009 than in 2009-2010 (figure 2b, page 23) ($H_{(2,\ N = 15)}$ = 6.46; P = 0.039).

The parasitoid impact was also similar between crops (figure 2c, page 23) (F = 0.01; df = 1, 6; P = 0.975), but different between growing seasons for soybean (F = 4.42; df = 4, 35; P = 0.005), being lower in 2008-2009 than in 2006-2007 and 2009-2010. For alfalfa this index did not vary between growing seasons (F = 1.54; df = 2, 17; P = 0.243).

The percentage of predated *P. guildinii* egg masses was low in soybean and alfalfa (1.02% and 3.51%, respectively). However, predation by chewing insects was very important in red clover, accounting for 61% of observed egg masses. Remains of approximately 33 chewed egg masses were collected. These egg masses could not be considered in the estimation of parasitism because they were significantly destroyed.

DISCUSSION

The spatial and temporal records found corresponds to what was expected since it is known that *P. guildinii* develops a first generation on this host plant after hibernation, feeding on alfalfa pods. During the 2008-2009 and 2009-2010 growing seasons weather conditions could have affected their populations directly and/or through the plant, and this would explain the relative time lag observed in the stink bug abundances. In 2008- 2009 there was a poor crop development due to the unusually low rainfall recorded mainly during December 2008 (10 mm in relation to the averaged 100 mm) and January 2009 (14.5 mm in relation to the averaged 120 mm). This could have also affected the alfalfa crop in the following growing season because the drought persisted until July 2009 and the crop was not harvested or irrigated. In addition, during February 2010 while alfalfa was mainly on the flowering stage, rains were unusually abundant (321 mm in relation to the averaged 117 mm) resulting in flooding problems.



Different capital letters indicate significant differences among growing seasons, for a given crop. Different lowercase letters indicate significant differences between crops, during a given growing season.

Letras mayúsculas distintas indican diferencias significativas entre las temporadas, para un cultivo dado. Letras minúsculas distintas indican diferencias significativas entre cultivos, para una temporada dada.

Figure 2. Discovery efficiency (a), parasitism efficiency (b) and parasitoid impact (c) of parasitoids of *P. guildinii* egg masses in soybean and alfalfa, in different growing seasons.

Figura 2. Eficiencia de descubrimiento (a), eficiencia de parasitismo (b) e impacto del parasitoide (c) de los parasitoides de posturas de *P. guildinii* en soja y en alfalfa, en diferentes temporadas.

Although a smaller number of egg masses was recorded in red clover, this crop is important as a resource for *P. guildinii* when hibernation ends and soybean is not yet at the appropriate phenological state for this stink bug.

The parasitoid discovery efficiency (DE) of *P. guildinii* eggs was variable in different growing seasons both in soybean and in alfalfa, being particularly low in 2007-2008 and 2008-2009. In these same growing seasons parasitism efficiency (PE) was the highest recorded, but the low number of egg masses found ameliorated this effect, leading to an overall low impact of parasitism (IP), especially in soybean in 2008-2009. The low parasitism of 2007-2008 and 2008-2009 corresponded to very dry years. During January and February 2008 rains were somewhat scarce and highly variable in the region, causing major limitations in the region east of Buenos Aires, where this study was conducted. In December 2008 and January 2009, the major drought was aggravated by several consecutive days of high temperature (around 30°C) and low humidity, which had a desiccating effect. This caused significant outbreaks of insects associated with dry conditions, such as grasshoppers, whiteflies, thrips and spider mites (22), and reduced soybean yields to 10-15 quintals/ hectare (5) in relation to yields of 30-35 quintals/ hectare expected for the region (34). Exposure to extreme temperatures can induce lethal or sublethal effects in parasitoids, reducing longevity, fertility, mobility and the ability to orient toward odor sources (15). Furthermore, the performance of platygastrids is very low or even null at temperatures above 30°C (16, 27).

In relation to egg parasitism, Ribeiro & Castiglioni (30) also found high rates of parasitism of *P. guildinii* caused by *T. podisi* (99.65%) and low parasitism by *T. basalis* (0.04%) in soybean fields in Uruguay. These authors reported the occurrence of *Trissolcus brochymenae* (Hymenoptera: Platygastridae), a species not found in this study. In contrast, parasitism of *P. guildinii* eggs in Londrina (Paraná State, Brazil) was mainly caused by *T. basalis*, followed by *T. podisi*. High parasitism of this stink bug's eggs by the former would have been favored by the increased abundance of this parasitoid, caused by the frequent mass releases of these wasps conducted in the area (9). In Brazil the emergence of *T. brochymenae* and *Gryon obesum* (Hymenoptera: Platygastridae) from egg masses of *P. guildinii* was also observed, albeit in a low frequency.

Concerning the predation of egg masses, 33 of 54 egg masses of *P. guildinii* found in red clover over the three growing seasons had been preyed upon, representing 61% of the total observed. Moreover, during sampling, larvae and adults of several species of predatory insects (ladybirds, lacewings and ants) were observed.

With respect to the emergence of two species of parasitoids from an individual pentatomid egg mass, 30 found one egg mass (out of 285 collected egg masses) from which individuals of both *T. basalis* and *T. podisi* emerged. These results suggest that the emergence of two or more species from the same egg mass is not common, although it is impossible to determine if it corresponds to a low frequency of multiparasitism, or to strong competitive interactions.

Field results suggest little relevance of *N. viridula* as an alternative host for the parasitoid species that attacked *P. guildinii* eggs. Egg masses of two other common pentatomid species (*Edessa meditabunda* (F.) and *Dichelops furcatus* (F.)) were found parasitized by *T. urichi* during the present study, although in a small number.

Laboratory experiments showed a very good performance of *T. urichi* when using eggs of *P. guildinii* as host (8). However, the field parasitism of this host-parasitoid combination did not reflect the laboratory results. The set of species that can support development of *T. urichi*, or its fundamental host range, observed under laboratory conditions was different than its ecological host range, *i. e*. the set of host species actually used for successful reproduction in the field (26). Many studies have shown that the fundamental host range of a biological control agent is often greater than its ecological host range (25). This is likely due to difficulty in accurately reproducing the factors that influence host searching and assessment behavior of a parasitoid in its natural environment (31). During our field surveys, *D. furcatus* was available for *T. urichi* in the surrounding natural vegetation, which was not sampled. On the other hand, in the 2010-2011 growing season, *T. urichi* would have taken advantage of the increased availability of *P. guildinii* due to its greater abundance relative to *D. furcatus*. In the province of Córdoba *D. furcatus* is a more frequent stink bug than *P. guildinii* and *T. urichi* was only found parasitizing egg masses of the former and of *E. meditabunda* (24).

The host range of a parasitoid species is determined in part by the searching and selection abilities of female wasps, as well as by their performance (*i. e.*, reproductive success and survival), as a function of the quantity and quality of available hosts (14). Therefore, the efficiency of parasitoids in detecting host egg masses (*i. e.*, the proportion of parasitized egg masses) and their parasitism efficiency (*i. e.* the proportion of parasitized eggs per attacked egg mass) will determine the overall impact of parasitism. *P. guildinii* has been mentioned as a host for *T. urichi* (7). Even though this parasitoid was recovered from *Edessa meditabunda* and *Dichelops furcatus* egg masses in the studied area (8), *T. urichi* only parasitized *P. guildinii* egg the year the abundance of this stink bug was greater. *T. urichi* could be a potential biological control agent of *P. guildinii* in the region, especially when this stink bug is more abundant.

CONCLUSIONS

Piezodorus guildinii was dominant in all three crops, and *N. viridula* egg masses were collected only in soybean and its abundance was much lower.

Piezodorus guildinii egg masses were parasitized by *T. podisi*, *T. urichi* and *T. basalis* in soybean and in alfalfa, while no parasitism was registered in red clover. *N. viridula* was parasitized only by *T. basalis*.

Telenomus podisi was the predominant parasitoid of *P. guildinii* egg masses. Parasitism by this species was recorded during all the studied growing seasons, both in soybean and in alfalfa. *Trissolcus urichi* was recorded in both crops, but only in 2010-2011, when *P. guildinii* eggs abundance was greater.

In turn, *T. basalis* accounted only for 6.77% of the wasps emerged from *P. guildinii* eggs.

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