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Potential of *Hexacladia smithii* (Hymenoptera Encyrtidae) to parasitize *Piezodorus guildinii* (Hemiptera Pentatomidae) adults

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Abstract

Piezodorus guildinii (Westwood) (Hemiptera Pentatomidae) is an important pest of several crops. Biological control using parasitoids as natural enemies of pests represents a promising alternative strategy to the use of pesticides. Oophagous parasitoids are helpful in the management of this pest, but they are not sufficient for the control of the target insect. The aim was to assess the potential of a parasitoid of the adult stage of related stink bug species, *Hexacladia smithii* Ashmead (Hymenoptera Encyrtidae), as a biocontrol agent targeting *P. guildinii.* In the laboratory, adults of this stink bug were successfully parasitized, allowing also the emergence of a first filial generation of parasitoids. The percentage of emergence of wasps reached 61.9%. The results obtained indicate that *H. smithii* has the potential to develop in this host. This is the first record of *H. smithii* developing two generations on *P. guildinii* adults, indicating that this stink bug is a component of the host range of *H. smithii*. The action of parasitoids that attack the adult stage of hosts, such as *H. smithii*, in combination with the control exerted by the egg parasitoids, may be useful for the management of *P. guildinii*.

Key words: host range, integrated pest management, natural enemies, performance, stink bugs.

Introduction

Phytophagous hemipterans that belong to the family Pentatomidae, commonly known as stink bugs, are important pests of several crops. From the whole complex of pentatomids that attack soybeans in the Americas, the redbanded stink bug *Piezodorus guildinii* (Westwood) provokes the deepest seed damage affecting greatly the quality and viability of seeds. It also causes greater leaf retention which affects the harvesting process, when compared to *Nezara viridula* L. and *Euschistus heros* (F.) (Depieri and Panizzi, 2011). This Neotropical multivoltine species is highly mobile and more difficult to control as it is less susceptible than other stink bugs to insecticides (Temple *et al*., 2013).

P. guildinii is one of the predominant stink bugs and a serious pest of soybeans in several states of the south of the United States of America, Brazil, Uruguay and Argentina. In the last two decades *P. guildinii* relative abundance has been increasing significantly and has currently become the most important species in several provinces of Argentina. It has been identified also as potential invasive species for Europe through pest risk analysis (Bundy *et al*., 2018).

Stink bugs are attacked by a diverse complex of natural enemies, being parasitoids of the eggs and those of adults the most effective. Among them, egg parasitoids (Hymenoptera Platygastroidea) are very common and may lead to efficient local regulation of stink bug populations (Zerbino and Panizzi, 2019). Several species of flies (Diptera Tachinidae) and microhymenopterans (Hymenoptera Encyrtidae) parasitize adults of all known stink bug species in the complex of soybean pests, except *P. guildinii* (Liljesthröm and Ávalos, 2015), which represents an empty niche. From the early 1970s, the cosmopolitan species *N. viridula* was the predominant soybean pest in the Neotropical region, but from 2000 onwards, it has sharply decreased in abundance (Panizzi and Lucini, 2016). On the contrary, in the last two decades *P. guildinii* relative abundance has been increasing significantly and it has currently become the most important species in several provinces of Argentina. The lack of known adult parasitoids attacking *P. guildinii* may contribute to the explanation of its current expansion and pest status (Panizzi and Lucini, 2016).

Nowadays soybean stink bugs are controlled worldwide using large amounts of insecticides of variable effectiveness. Products with a broad spectrum of action as pyrethroids and organophosphates are used in high doses and in repeated applications (Conti *et al*., 2021), causing a negative impact on the environment and, in particular, non-target species (Bernasconi *et al*., 2021). Biological control through the use of entomophagous insects antagonist of insect pests is a promising alternative strategy that may help reducing the use of conventional insecticides and their negative effects on soybean agroecosystems, in the framework of integrated pest management. Parasitoids of the adult stage of stink bugs are characterized by reducing the longevity and reproductive capacity of their host, as well as the amount of damage that their host can cause to the crop. For example, Nunes and Corrêa-Ferreira (2002a; 2002b) found that parasitism caused by *Hexacladia smithii* Ashmead (Hymenoptera Encyrtidae) to *E. heros* drastically reduced (5.2 folds lower) host's fecundity.

Species of the genus *Hexacladia* are endoparasitoids of adult hemipterans (Pentatomidae, Coreidae, Pyrrhocoridae and Scutelleridae) (Torréns *et al*., 2018). In particular, *H. smithii* is a gregarious parasitoid whose larval and pupal stages develop inside the host, completing their cycle in approximately 35 days following parasitism. After that, adults emerge through holes made in the ventral or dorsal side of the abdomen of the host. This parasitoid is recorded in Brazil parasitizing adults of several pentatomid species, as *N. viridula*, *P. guildinii* and *E. heros* (de Aquino, 2016; Carneiro *et al*., 2010). In Argentina, *H. smithii* was recorded for the first time in 1997 emerging from *E. heros* and *Antiteuchus variolosus* Westwood (Hemiptera Pentatomidae) (Cuezzo and Fidalgo, 1997), and 20 years later Torréns *et al*. (2017) recorded this parasitoid species emerging from *Diceraeus furcatus* (F.), its preferred host.

The overall knowledge of the taxonomy, biology and ecology of parasitoids of adult stink bugs is scarce and fragmented (Dindo, 2011; Zerbino and Panizzi, 2019). As regards *P. guildinii*, to the best of our knowledge, the only findings in nature were the unusual occurrence of adult parasitized by the tachinid flies *Trichopoda pennipes* (F.) in Brazil (Panizzi and Slansky, 1985) and *Euthera tentatrix* Loew in the United States of America (Buschman and Whitcomb, 1980).

Our aim was to increase information on *H. smithii* and to assess its potential as a biocontrol agent targeting *P. guildinii,* considering that this parasitoid attacks the adults of related stink bug species. Moreover, a record of *H. smithii* parasitizing *P. guildinii* was mentioned by Bollati *et al*. (2018), who performed some preliminary laboratory choice tests and recorded adult parasitoids (only two) emerging from this host. Also, this study encouraged us to go further with the investigations on the association occurring between *P. guildinii* and *H. smithii.* Improving knowledge of natural enemies that may reduce phytophagous populations is a necessity for the development of efficient integrated pest management programs.

Materials and methods

Colonies of stink bugs and wasps were established from individuals collected in a soybean plot at the Experimental Station "Julio Hirschhörn" of the Faculty of Agricultural Sciences and Forestry of the National University of La Plata, Argentina (34°59'24.32"S 58°0'18.51"W; 27 m a.s.l.). The laboratory rearing of *H. smithii* originated from wasps emerged from adults of *D. furcatus* collected during the 2018-2019 soybean growing season, and wasps' species identity was determined by one of the authors of this work (DAA), following Noyes (2010) and Torrens *et al*. (2017), and compared with voucher material deposited in the Entomology Division of the La Plata Museum. Voucher specimens were stored in the laboratory of Pest Ecology and Biological Control of CEPAVE (CONICET-UNLP-Asoc CICPBA). Adult wasps were maintained in rearing cages at 24 ± 1 °C, 70 ± 10% RH and a photoperiod of 14:10 (L:D), with adults of *D. furcatus* as hosts and honey drops as food.

Adults of *P. guildinii* and *D. furcatus* were fed *Phaseolus vulgaris* L. (Fabales Fabaceae) pods, and maintained at 24 ± 1 °C, 70 ± 10% RH and a photoperiod of 14:10 (L:D). Stink bugs rearing cages were visually inspected daily until the emergence of adult wasps. Newly emerged wasps were used for the experiments.

The experimental units consisted of an adult *P. guildinii* with a couple of adult *H. smithii*, inside a plastic container (10 cm diameter × 20 cm high) covered with a piece of voile fabric. Hosts were exposed to parasitoids for 48 hours. Pods and honey drops were used as food for stink bugs and wasps, respectively. The experimental units were kept under the same laboratory conditions mentioned above, until the death of stink bugs and wasps. It was performed two set of experiments (treatments) to evaluate the parasitism on *P. guildinii* using *H. smithii* wasps coming from different origins: 1) wasps emerged from *D. furcatus* parasitized individuals (Hs ex Df) (n = 26), and 2) wasps emerged from *P. guildinii* parasitized individuals (Hs ex Pg) (n = 21) originated from an incipient laboratory rearing of the parasitoid maintained on this host. All dead hosts were dissected and examined under stereomicroscope to evaluate the existence of dead parasitoids inside their body. In all cases we recorded the number of parasitized stink bugs: individuals from which emergence of adult wasps was observed, plus individuals with dead pupae and/or adult wasps inside their body. Besides, the date of death of stink bugs and wasps was recorded.

Results

Eight of the 26 (30%) adults of *P. guildinii* exposed in the Hs ex Df treatment, and two of the 21 (9%) *P. guildinii* exposed in the Hs ex Pg treatment were parasitized. From almost all the parasitized hosts in Hs ex Df treatment (7 out of 8) produced adult wasps emerged. In the other treatment (Hs ex Pg), 1 of the 2 parasitized hosts produced adult wasps.

The percentage of emergence of adult wasps, estimated as the number of emerged wasps from the total of developed wasps (the total of adult wasps emerged, plus the total of dead larvae, pupae and/or adult wasps inside the host body) was 90% (45 emerged wasps out of 50 wasps completely developed inside the host body) for the progeny from Hs ex Df treatment. This percentage was somewhat lower (61.9%, 13 out of 21) for the progeny from Hs ex Pg treatment. Wasps completed their preimaginal development (from egg to adult emergence) in 41 (± 2) and 38 days on average for progeny from Hs ex Df and Hs ex Pg treatments, respectively. All hosts died in the moment of wasp emergence, and all the emerged wasps died in around 5 days after emergence.

The maximum number of parasitoids per host that we recorded from one single stink bug reached a total of 19 wasps, from a host exposed to the Hs ex Df treatment. In all the cases in which parasitism was recorded (stink bugs from which adult wasps emerged, or dead larvae, pupae and/or adult wasps were observed inside the stink bug body), we noticed that the host's body turned yel-

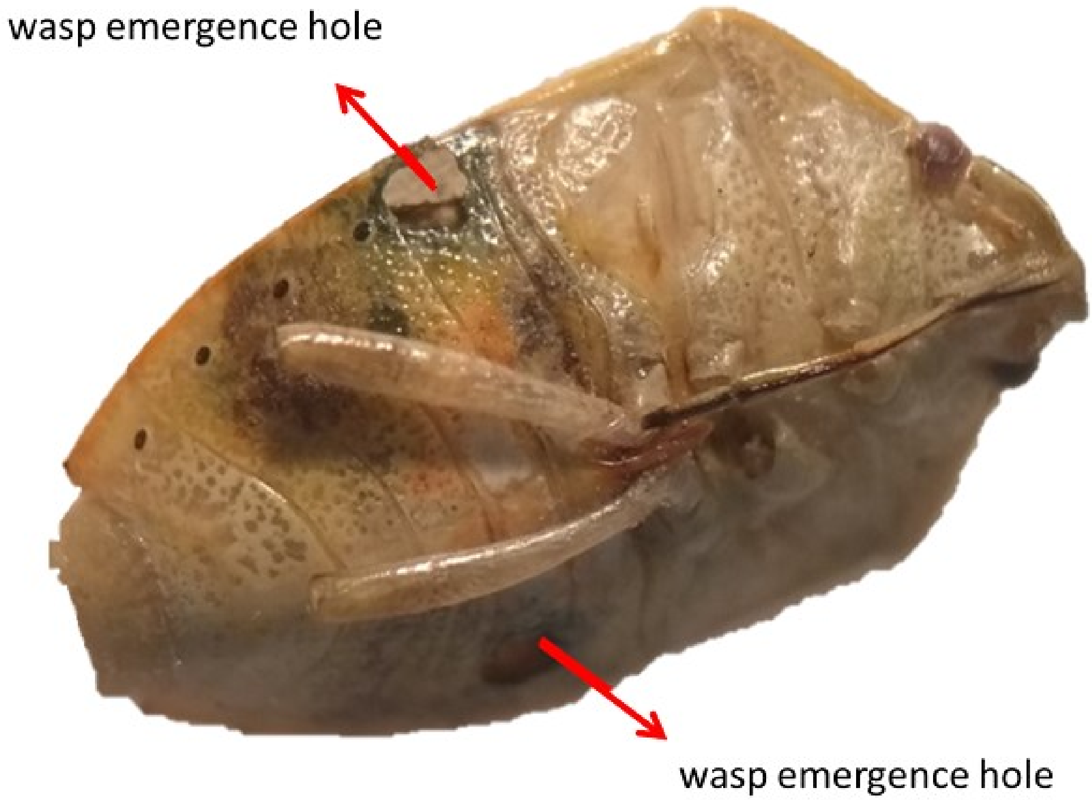

Figure 1. Ventral view of a parasitized adult of *P. guildinii* evidencing holes made by emerged *H. smithii* wasps.

lowish in coloration, with dark spots in the ventral side of the abdomen, near the lateral edges, denoting the presence of the endoparasitoid. One or two holes, chewed by the emerging adult wasps, were observed on the host body (figure 1). In some dissected hosts, we found a few wasps completely developed and dead inside the host's body (figure 2).

Discussion and conclusions

Although *P. guildinii* is not mentioned in the literature as a host for *H. smithii*, it was found that some adults of this stink bug were successfully parasitized, allowing also the emergence of a first filial (F1) generation of wasps. In this work it was reported that several individuals of *H. smithii*

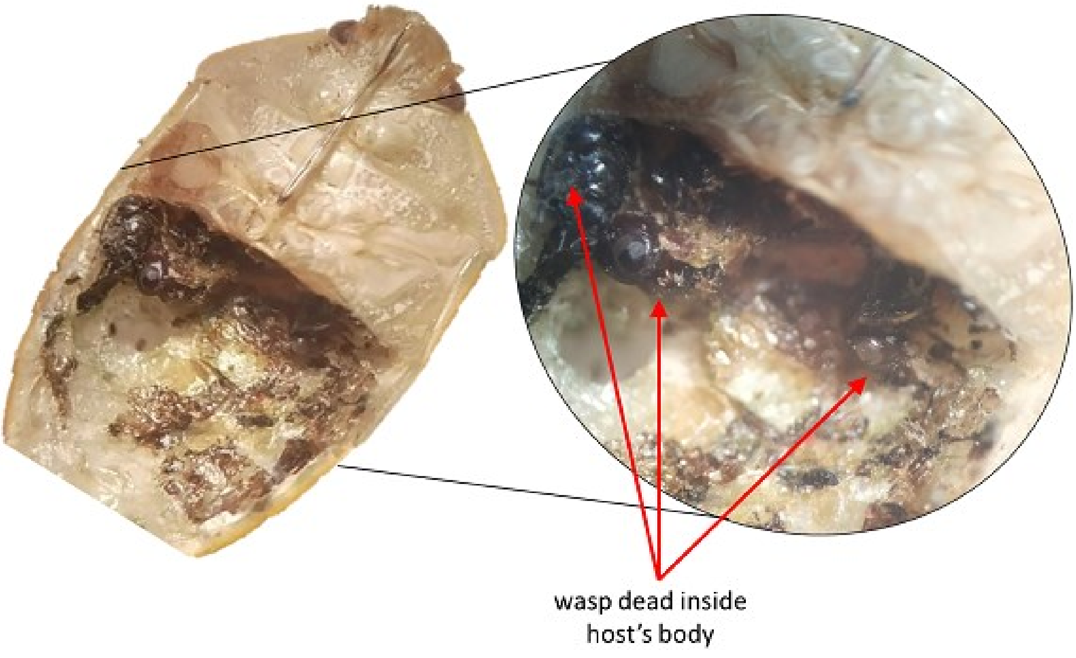

Figure 2. Dissection of parasitized adult of *P. guildinii* denoting adults of *H. smithii* dead inside the stink bug body.

successfully completed their life cycles in adults of *P. guildinii*, producing a viable first filial generation. These results indicate that *H. smithii* has the potential to parasitize successfully this host, being the first record of this parasitoid species developing for two generations in *P. guildinii* adults. Therefore, we suggest that this stink bug may be included among the components of the fundamental host range of *H. smithii*.

No progeny was produced by parasitized stink bugs; however, we cannot affirm that this phenomenon was a direct negative effect of parasitism, as we did not carry out further analysis of reproductive structures of parasitized female stink bugs. Future studies may address this topic.

Regarding *H. smithii*, the development time we recorded for this parasitoid (38-41 days) agrees with that expressed by Corrêa-Ferreira *et al*. (1998) for the same parasitoid species developing in *E. heros* (35 days), one of its preferred hosts. Moreover, the symptoms of parasitism that we recorded are also consistent with those described in other works that detailed the effects of the parasitism on other pentatomid hosts (Panizzi and Silva, 2010).

Corrêa-Ferreira *et al*. (1998) reported a reduction in the reproductive potential of the *E. heros* adults parasitized by *H. smithii*, and also that adults stink bugs were less active and caused less damage to soybean (Nunes and Corrêa-Ferreira, 2002a; 2002b). Therefore, the action of parasitoids that attack the adult stage of hosts, such as *H. smithii*, in combination with the biological control exerted by egg parasitoids, can be very beneficial for the management of the target pest species. In one hand, egg parasitoids prevent the emergence of their hosts; in the other hand, parasitoids of the adult stage of pests contribute to minimize their impact to the crop, as they limit their reproductive potential and the feeding damage they can cause.

An interesting peculiarity of *P. guildinii* is that it is not commonly attacked by parasitoids of the adult stage (Liljesthröm and Ávalos, 2015), and its population's density is increasing more rapidly than the other species of the soybean phytophagous hemipteran complex (Panizzi and Lucini, 2016), which are attacked by these parasitoids. We wish to highlight that *H. smithii* accepted *P. guildinii* adults as alternative hosts inducing some mortality and probably impacting on host's fecundity (i.e. reduction of maximum potential reproductive output of parasitized individuals). For this reason, it is very important to take in consideration this parasitoid as another natural enemy of *P. guildinii*, and to continue evaluating the impact of parasitism on the reproductive capacity and other biological traits of the host.

Some other parasitoids of the adult stage of pentatomids, like tachinid flies, can reduce the potential longevity of adult hosts, giving an important advantage to control these pests (Liljesthröm, 1993). Parasitism reduces the host's longevity and therefore causes a reduction in the period during which the host can damage the crop.

As mentioned above, *P. guildinii* has not developed yet any stable interaction over time with parasitoids of the adult stage in the field, nor with encyrtids or with tachinids. A better understanding of the aspects that may be determining this phenomenon is needed. Many are the factors that influence the host acceptance. For example, young egg parasitoid wasps *Trissolcus basalis* (Wollaston) (Hymenoptera Platygastroidea) did not parasitize *P. gulidnii*, a not preferred host. However, wasps that were deprived of hosts as they aged becoming time-limited, accepted *P. guildinii* eggs, having also a good performance on this alternative host (Cingolani *et al*., 2014). It is well known that parasitoids may overtime adapt to new hosts (Ferreira Santos de Aquino *et al*., 2012; Abram *et al*., 2017) which could eventually allow improving their potential as biological control agents. Moreover, genetic variations can be exploited by selecting and breeding only individuals with the desired characteristics, via strain selection or selective breeding (Kruitwagen *et al*., 2018). The results obtained in this study give preliminary information about the association between *H. smithii* and *P. guildinii* adults which can be useful for the exploration of those techniques. Besides, many parasitoids display host preferences according to different chemical cues and experience (i.e. previous contacts with the host) (Peri *et al*., 2011). A parasitoid female could prefer the same host species from which it emerged over other host species. Several authors suggested that the host preference pattern of parasitic insects may be altered by preimaginal conditioning (Giunti *et al*., 2015). It would be interesting to explore whether females of *H. smithii* emerged from *P. guildinii* exhibit preference towards this host, making parasitism more efficient. This aspect is of particular interest in the case of inoculative biological control programs, in which the control is due not only to the released organisms themselves but also to their progeny (Hajek and Eilenberg, 2018).

Parasitoids are also capable of learning and memorizing certain stimuli by the acquisition and retention of neuronal representations of new information (Giunti *et al*., 2015). Some female parasitoids could improve their performance based on the knowledge acquired during the larval stage (Giunti *et al*., 2015). This shows that, in the case of generalist parasitoids, new hosts could be included as acceptable hosts if the female includes a new interaction in her experience, and this could be the case of *H. smithii* and *P. guildinii*.

Many are the unknowns that still remain around the interactions of *H. smithii* with its multiple potential hosts. Further works with this parasitoid will allow us to better understand whether some of these interactions are simply occasional occurring only sporadically or under certain conditions, or if they are stable interactions which may be useful in the field of applied biological control. It is also crucial to explore how well established *H. smithii* is in our study area. Since we reported the species for the first time in the 2018-2019 soybean growing season, it was not recovered again from any of their usual hosts in the region, despite the fact that *D. furcatus* and *Edessa meditabunda* F. (Hemiptera Pentatomidae) adults were collected regularly. Corrêa-Ferreira *et al*. (1998) observed a drastic reduction in *H. smithii* population density after recording high parasitism rates in Paraná State (Brazil), which authors linked to the absence of the host in the field. As it is known the presence of alternative hosts is crucial for parasitoid survival when the main hosts are scarce or unavailable in the field. The results obtained in this work indicate that *P. guildinii* may be a good alternative host for *H. smithii*, also through generations, and thus can contribute to the persistence of the parasitoid in the field while its preferred host is scarce. This could represent a useful alternative in the framework of conservation biological control.

However, although in these experiments *H. smithii* located and accepted *P. guildinii* adults as hosts, it is important to consider that our experimental arenas only represented a microcosm in which parasitoids were in near proximity of the stink bug. In the field, the parasitoid needs to seek a variety of cues to be guided towards the host. These include chemical and visual stimuli that the parasitoid must efficiently explore, while dealing with other abiotic and biotic factors. Further study of the behaviour of *H. smithii* in relation to these cues could help to explain the low encounter rates between this parasitoid and *P. guildinii* in the field. Since each parasitized host is killed, parasitoid searching efficiency and resource holding potential is crucial to parasitoid-host population dynamics, and therefore to the exerted biological control (Godfray, 1994).

Although chemical pest control is the predominant management strategy in Argentina and other countries in South America, it is not very effective with regard to stink bugs. Particularly, it is known that *P. guildinii* is more resistant to some pesticides than other stink bugs of soybean complex (Temple *et al*., 2013). This fact demonstrates the importance of seeking alternative ways to control this pest that favour the persistence of spontaneous natural enemies in the environment. Biological control is a promising alternative to be implemented although it still remains at a low level of adoption and its high potential is generally underestimated (Zerbino and Panizzi, 2019).

Being *H. smithii* a generalist parasitoid (Turchen *et al*., 2015) it is possible that it has a better ability to adapt to non-preferred hosts than specialist ones. Nonetheless, the interaction of *H. smithii* with its well-known and potential hosts is still not deeply known. More laboratory and field studies are needed, not only targeting crops where pests are present but also spontaneous vegetation surrounding fields, where alternative hosts for the parasitoid can be found. In this sense, the evaluation of strategies that enhance the conservation of natural populations of parasitoids is essential to improve pest suppression, a valuable ecosystem service.

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