Bulletin of Entomological Research (2008) **98**, 203–206
doi:10.1017/S0007485307005548

Printed in the United Kingdom

Survey of *Neodohrniphora* spp. (Diptera: Phoridae) at colonies of *Atta sexdens rubropilosa* (FOREL) and specificity of attack behaviour in relation to their hosts

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Abstract

Atta sexdens rubropilosa is a leaf-cutting ant that is a significant agricultural and forestry pest in the Neotropical region. This ant is parasitized by flies from the genera *Neodohrniphora* spp., *Apocephalus* spp. and *Myrmosicarius* spp. This study was carried out to determine which species of *Neodohrniphora* spp. are found near foraging trails of *Atta sexdens rubropilosa* and to evaluate the specificity of attack behaviour of these parasitoids. From May 2002 to April 2004, we sampled *Neodohrniphora* spp. hovering over foraging trails of *Atta sexdens rubropilosa* between 8:00 and 11:00 h and between 15:00 and 18:00 h. To investigate the attacking behaviour against the ants, flies were released individually inside an observation chamber containing a single leaf-cutting ant worker. Each parasitoid was confronted successively with a worker ant of *A. sexdens rubropilosa*, *Atta laevigata* Smith, *Acromyrmex crassispinus* Forel and *Acromyrmex subterraneus molestans* Santschi. Phorids of three species were identified: *Neodohrniphora elongata* Brown, *Neodohrniphora declinata* Borgmeier and *Neodohrniphora tonhascai* Brown. The three phorid species were active throughout the year and often along the same foraging trails, but *N. elongata* was the most frequent species. In the laboratory assay, *N. elongata*, *N. declinata* and *N. tonhascai* attacked workers of *A. sexdens rubropilosa*, *A. laevigata* and *A. crassispinus*, but not of *A. subterraneus molestans*.

Keywords: *Acromyrmex* spp., *Atta* spp., phorids, attack behaviour, parasitism

(Accepted 4 July 2007)

Introduction

The leaf-cutting ants *Atta* spp. and *Acromyrmex* spp. are hosts for 38 species of parasitoids from the Phoridae family, mainly from the genera *Neodohrniphora* spp., *Apocephalus* spp. and *Myrmosicarius* spp. (Feener & Moss, 1990; Disney,

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1996; Disney & Bragança, 2000; Brown, 2001; Disney *et al.*, 2006). These parasitoids can be observed flying along foraging trails of the ants (Borgmeier, 1928, 1931; Disney, 1996; Disney & Bragança, 2000; Brown, 2001).

Atta sexdens rubropilosa, which is an important agricultural and forestry pest in the Neotropical region, is frequently parasitized by *Neodohrniphora* spp. (Diptera: Phoridae) (Fowler *et al.*, 1989). Parasitism levels of these flies are relatively low, but their presence over nests and trails also modifies the ants' behaviour and reduces the foraging ability of the colonies (Orr, 1992; Feener & Brown, 1993; Bragança *et al.*, 1998).

Neodohrniphora spp. are suggested as potential agents of biological control of ants (Tonhasca, 1996; Bragança *et al.*, 1998); however, little is known about their host specificity and diversity (Brown, 2001). In the few studies where the *Neodohrniphora-A. sexdens rubropilosa* interaction was investigated, the identification of flies was limited to the genus level (Tonhasca, 1996; Bragança *et al.*, 1998).

The morphology of *Neodohrniphora* species is similar, except for their ovipositors, which apparently are modified in relation to theirs hosts (Brown, 2001). Nonetheless, there is evidence that some species parasitize more than one species of leaf-cutting ant (Brown, 2001; Bragança *et al.*, 2002). In the work reported here, we surveyed the occurrence of species of *Neodohrniphora* phorids in the proximity of *A. sexdens rubropilosa* nests; and, on the basis of the attacking behaviour of phorids, we discuss the possibility that these flies parasitize other *Atta* and *Acromyrmex* species.

Material and methods

Phorid sampling

Phorids were sampled two to four times every month between May 2002 and April 2004 in a wooded patch located in Viçosa (20° 45′ S; 42° 51′ W), Brazil. Ten *A. sexdens rubropilosa* nests were initially selected on the basis of accessibility of foraging trails and abundance of forager ants. Sampling was conducted between 8:00 and 11:00 h and between 15:00 and 18:00 h. An observer walking at a constant pace captured flies hovering over the trails using a glass tube. The specimens were prepared in the laboratory and identified with the most recent key (Brown, 2001).

To analyze if there is a variation in the phorids occurrence throughout the year, we compared the number of phorids collected by bimesters (the monthly data were grouped into bimesters because two monthly samplings were lost). The data were analyzed using the Kruskal-Wallis test because they were not normally distributed.

Attack behaviour specificity

To investigate the specificity of attack behaviour, females of *Neodohrniphora* spp. were released in a glass chamber ($50 \times 50 \times 50$ cm) containing a single worker ant and filmed for five minutes. Four ant species were tested consecutively with each phorid: *A. sexdens rubropilosa*, *Atta laevigata*, *Acromyrmex crassispinus* and *Acromyrmex subterraneus molestans*. The phorids that showed no response to the four ant species were not used in the analysis. The order of ant species in the test was randomized for each phorid tested ($n = 20$). After the tests, the flies were killed and identified at the species level. The tapes were analyzed with help of the software, The Observer, version 4.1 (Noldus Information Techonology). We considered three behavioural acts associated with host attack: inspection flights, attacking bouts and attacks (Orr, 1992; Feener & Brown, 1993; Silva *et al.*, 2007). Inspection flight was considered when the phorids hover at no more than 5 cm above a host. Attacking bouts are when the phorids make contact with any body part of the host without ovipositor penetration. Finally, attacks are contacts with ovipositor penetration in the posterodorsal extremity of the ant's head.

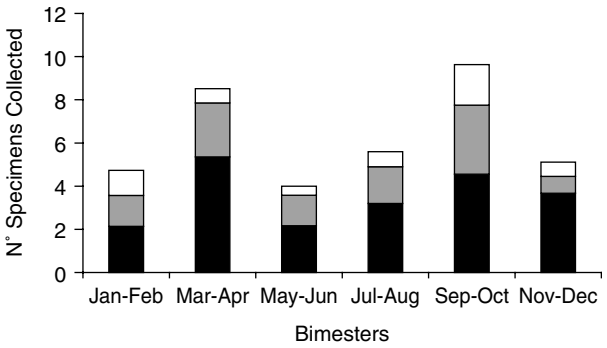

Fig. 1. Daily mean number of individuals of three *Neodohrniphora* species in Viçosa, Brazil (□, *N. tonhascai*; ▩, *N. declinata*; ■, *N. elongata*).

Results

We collected 303 flies of the genus *Neodohrniphora*. Fifteen were males and, therefore, could not be identified at the species level. These numbers underestimated the actual abundance of phorid because the rate of capture was about 60% (phorids captured/phorids pursued × 100).

The phorids females (288 individuals) belonged to three species: *Neodohrniphora elongata* Brown (54% of individuals), *Neodohrniphora declinata* Borgmeier (31%) and *Neodohrniphora tonhascai* Brown (15%). Individuals of each phorid species were observed attacking ants of *A. sexdens rubropilosa* in the foraging trails.

The three phorid species were found throughout the year and did not differ in seasonal patterns of activity (fig. 1), but the mean daily number of *N. elongata* (3.32 ± 0.34) was higher

Table 1. Attacking bouts (A.bouts) and attacks (means ± s.e) carried out by trhee phorids species in four species of the leaf-cutting ants.

Phorid species	*A. sexdens rubropilosa*			*A. laevigata*			*A. crassispinus*			*A. subterraneus molestans*		
	n	A.bouts	Attacks	*n*	A.bouts	Attacks	*n*	A.bouts	Attacks	*n*	A.bouts	Attacks
N. elongata ($n = 12$)	9	1.7 ± 0.9	1.0 ± 0.3	9	2.1 ± 0.8	1.2 ± 0.3	6	1.2 ± 0.5	0.7 ± 0.3	0	–	–
N. declinata ($n = 4$)	3	1.3 ± 1.4	1.5 ± 0.5	4	1.3 ± 0.5	1.0 ± 0	1	1.3 ± 0.5	0.3 ± 0.3	0	–	–
N. tonhascai ($n = 4$)	4	0.5 ± 1.2	1.3 ± 0.5	2	3.3 ± 0.6	1.0 ± 0.6	4	0.7 ± 0.3	2.0 ± 0.5	0	–	–

than for *N. declinata* (1.77 ± 0.30) and *N. tonhascai* (0.87 ± 0.14) ($\chi^2 = 14.60$; $P < 0.001$). The number of phorids captured did not differ significantly between the two years nor among bimesters ($P < 0.05$).

The three *Neodohrniphora* species were found together at 37% of the samplings. They were found during both sampling times (in the morning and in the afternoon). Two species were found at 36% of samplings (23% *N. elongata* and *N. declinata*; 12% *N. elongata* and *N. tonhascai*; 1% *N. declinata* and *N. tonhascai*). *N. elongata* was found alone in 19% of the samplings, *N. declinata* in 7%, and *N. tonhascai* in 1%.

In laboratory tests, inspection flights were observed for the three phorid species with the four ant species. The mean duration and the 95% confidence interval of the inspection flights of *N. elongata*, *N. declinata* and *N. tonhascai* were 60.47 s (45.29–75.65 s) ($n = 50$), 46.49 s (28.01–64.98 s) ($n = 16$), and 67.86 s (32.07–103.65 s) ($n = 14$), respectively. Except for *A. subterraneus molestans*, all ant species were attacked by the three phorid species (table 1). The mean frequency of attacking bouts and attacks on ants were 1.18 ± 0.25 ($n = 80$) and 0.76 ± 0.10 ($n = 80$), respectively. The mean duration and the 95% confidence intervals of attacking bouts and attacks were 0.32 s (0.18–0.47 s) ($n = 80$) and 0.92 s (0.58–1.26 s) ($n = 80$), respectively.

Discussion

Differences in the frequencies of phorids were not detected over the year. However, the largest numbers were captured in September–October, which represents the onset of the rainy season. Erthal (1999) found this seasonality for *Neodohrniphora* spp. attacking *A. laevigata*.

The higher frequencies of *N. elongata* observed may be due to either a relatively high abundance of this species in relation to *N. tonhascai* and *N. declinata* or to a higher preference of this parasitoid for *A. sexdens rubropilosa*, since *N. declinata* is described as a parasitoid of other ant species (Brown, 2001).

In laboratory tests, the confidence intervals of inspection flight durations suggest a similar host inspection mechanism for the three phorid species. The duration of attacking bouts was significantly lower than the duration for attacks, and the absence of ovipositor penetration during these bouts may indicate that this behaviour allows flies to test the suitability of the potential host.

The attacks recorded on different ant species show that the three parasitoid species can attack non-host leaf-cutting ants. However, this plasticity is restricted because the absence of attacking bouts and attacks after inspections by flies on *A. subterraneus molestans* indicates that *Neodohrniphora* spp. are not very selective to locating hosts, but they have other mechanisms for host-recognition. Morehead & Feener (2000) found similar results in relation to the phorid *Apocephalus paraponerae* (Formicidae: Ponerinae), which locate *Paraponerae clavata* and *Ectatomma ruidum* (Formicidae: Ponerinae) with equal efficiency but only attacks *P. clavata*. This plasticity in the host location mechanism probably serves to prevent premature rejection of individuals that belong to polymorphic host species.

N. declinata is described by Brown (2001) as a parasitoid of *A. laevigata*, but in this work we found females of this phorid all year long over foraging trails of *A. sexdens rubropilosa* in areas where there were neither trails nor nests of *A. laevigata*. Moreover, we observed that this parasitoid attacks these ants in field and laboratory conditions. Therefore, these results suggest that this phorid may be also a parasitoid of *A. sexdens rubropilosa*. However, to confirm this host-parasitoid relationship, it should be determined if a new generation of flies may be obtained from the attacked ants (Gilbert & Morrison, 1997; Porter, 1998; Porter & Alonso, 1999).

As well as *N. declinata*, *N. elongata* and *N. tonhascai* also have behavioural plasticity to attack other non-host ants, including other genera. This plasticity of attack behaviour of *Neodohrniphora* spp. has also been reported by Bragança *et al*. (2002), who verified, in laboratory conditions, parasitism of *N. tonhascai* on *A. laevigata* with phorids collected from *A. sexdens rubropilosa* trails. However, in these situations, it is difficult to suggest a host-parasitoid relationship because the attacks were only observed in a laboratory in a no choice situation, and we do not know if these phorids find the same ants in the field (Porter, 1998; Orr *et al*., 2001).

The data presented in this paper demonstrate that *N. declinata*, as well as *N. elongata* and *N. tonhascai*, was present all year long over foraging trails of *A. sexdens rubropilosa* and attacks this ant in laboratory and field conditions. Further investigations must be carried out to determine if such attacks of *N. declinata* also produce a new generation of phorid flies in order to prove a host-parasitoid relationship.

Acknowledgements

We thank Brian V. Brown for helping with phorid identifications, M. Bragança for suggestions to improve the manuscript, and T.R. Souza, Denise D.O. Moreira and A. de Fátima for helping with laboratory work. V.S.G. Silva was supported by a grant from FAPERJ. This research was supported by the International Foundation for Science.

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