Effect of phorid fly density on the foraging of *Atta vollenweideri* leafcutter ants in the field

Andrea C. Guillade* & Patricia J. Folgarait

Laboratorio de Hormigas, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal B1876BXD, Buenos Aires, Argentina

Abstract

Leafcutter ants in the genus *Atta* (Hymenoptera: Formicidae: Attini) are considered major pests of agriculture and forestry in the Neotropics. Phorid flies (Diptera: Phoridae) have been proposed as viable candidates for biological control of ants because of the importance of their trait-mediated effects on their hosts. However, the impact of different densities of phorid flies has never been assessed in the field. Experiments were conducted by isolating 3-m sections of *Atta vollenweideri* Forel foraging trails with tunnels, and sampling ants in trails with 0, 1, or 4 *Eibesfeldtphora trilobata* Disney female parasitoid flies. Samples were collected every 30 min from these trails. We also collected a sample before introducing the parasitoids and another one 30 min after removing them from the trail. We measured traffic of ants on the trails, weight and type of plant material transported, and the proportion and size of the workers collected. The presence of phorids on the trails reduced the ant traffic and amount of plant material transported into the nests and decreased the proportion of workers on the trails in the size range preferred as hosts by the flies. The effect on worker size, as well as the lag effect recorded after phorids were removed from the tunnels, was more pronounced with four phorids. The presence of phorids also affected the weight of monocotyledon and dicotyledon material transported. Even at the minimum density possible, phorids significantly influenced a key aspect of the colony life, the food intake through foraging. From an applied point of view, our results show that releases of these phorids into the field should not necessarily involve many individuals to reduce foraging by *A. vollenweideri*, making them potentially useful candidates for biological control of these ants.

Introduction

Leafcutter ants in the genus *Atta* (Hymenoptera: Formicidae: Attini) cut plant material from a wide variety of species to grow a symbiotic fungus, which in turn serves as a food source for the colony (Weber, 1972; Hölldobler & Wilson, 1990). This relationship has allowed the ants to circumvent most plant natural defense mechanisms (Cherret, 1986), and it is one of the reasons why leafcutters have become the dominant herbivores in the Neotropics (Hölldobler & Wilson, 1990). With the expansion of intensive agriculture and the implementation of monocultures, many species of leafcutter ants have become pests, and great efforts have been made over the years to eliminate them from agroecosystems (Cherret, 1986). Chemical control has been effective in the short term (Boaretto & Forti, 1997; De Coll, 1998; Filho & Dorval, 2003; Zanetti et al., 2003); however, some pesticides such as chlorpyrifos have been proven ineffective in long-term field studies (Guillade & Folgarait, 2014), whereas others have been banned from several crops. More effective pesticides like fipronil are currently scheduled to be forbidden by the Forest Stewardship Council for certifiable forestry within the next year or two, depending on the country (Forest Stewardship Council, 2007, 2012). Moreover, organic producers simply do not have the option to control leaf-cutting ants with synthetic pesticides (Foguelman, 2003). It is then essential that alternative strategies for the control of these pests are devised for these sectors.

Parasitoids in the Phoridae family (Diptera) have been the focus of much research over the past three decades (Folgarait, 2013), especially because of their current use as biological control agents of fire ants in the USA (Callcott

*Correspondence: E-mail: andreaguillade@gmail.com

et al., 2011; Plowes et al., 2011, 2012; Porter et al., 2011). The female of these flies inserts an egg into the body of a worker ant using her ovipositor. The larva feeds from the tissues of its host, eventually killing it at the time of pupation (Disney, 1994). Parasitism rates of foragers are often low (Tonhasca, 1996; Erthal & Tonhasca, 2000; Morrison & Porter, 2005; Guillade & Folgarait, 2011), with the exception of those reported by Elizalde & Folgarait (2011). As well as this direct mortality, these flies can produce large indirect effects on the ants, individually as well as on the whole colony (Feener & Moss, 1990; Elizalde & Folgarait, 2012). Leafcutter ants exhibit strong defensive behaviors to these flies, such as raising the head and opening their mandibles in an attempt to hunt the flies, or curving the body, with the abdomen touching the ground, to protect themselves from phorid attacks (Folgarait, 2013). Ants have also been observed abandoning their loads and rushing back into the nests, to the point that normal traffic in the foraging trail is drastically altered (Orr, 1992; Bragança et al., 1998; Tonhasca et al., 2001). Thus, it has been proposed that the presence of phorids has a substantial effect on colonies through interference with the normal supply of plant material to the colony. Leafcutter phorids can also trigger shifts in diel foraging patterns, so that ants switch to nocturnal foraging to avoid parasitism (Orr, 1992). Some species of leafcutter ant parasitoids are nocturnal as well (Elizalde & Folgarait, 2011). Several studies have shown that phorid flies can mediate competitive interactions between ant communities involving ants such as *Solenopsis* spp. (Feener, 1981; Morrison, 1999), *Pheidole* spp. (LeBrun, 2005; LeBrun & Feener, 2007), or *Azteca* spp. (Philpott, 2005), because they interfere with key aspects of the colony life, such as losing resources due to competition when foraging. Consequently, they have been called keystone parasitoids (Feener, 2000).

Several phorid species attack leafcutter ants in South America (Elizalde & Folgarait, 2010). *Atta vollenweideri* Forel is regarded as a monocotyledon cutter (Jonkman, 1976, 1980; Robinson & Fowler, 1982) that affects forestry and cattle ranches, although we have often observed them carrying dicotyledon items. Workers of this species have been reported to host six parasitoid species in Argentina (Elizalde & Folgarait, 2011; Guillade & Folgarait, 2011), including *Eibesfeldtphora trilobata* Disney, classified up to 2009 as *Neodohrniphora trilobata* Disney (Disney et al., 2009). This fly targets large hosts (2.1–2.5 mm head width) (Elizalde & Folgarait, 2011; Guillade & Folgarait, 2011), attacking worker ants in foraging trails near entrances and pupariating within the host's head (Elizalde & Folgarait, 2011). These flies select appropriate hosts from a perch, ambushing the ant as it walks along the trail. An attacking female lands on the ant's head to insert its

ovipositor in the occiput (Elizalde & Folgarait, 2012). Ants display a strong reaction to their presence on the trails, immediately assuming several defensive postures (Folgarait, 2013). They often run to escape phorids, and in this situation, being laden with grass fragments has been shown to decrease the running speed of foragers (Röschard & Roces, 2002), which would render them more vulnerable (Feener, 2000). On the other hand, a dicotyledon fragment may offer better protection to the ant by becoming an obstacle for the phorids when landing on the ant's head. Therefore, we hypothesized that attacking phorid females may alter the relative weight of monocotyledon and dicotyledon fragments carried into the nest.

Phorids in this genus disturb normal traffic to such an extent that, as noted by Bragança et al. (1998), it can be used as a sign that phorids are present on the trail. Such a conspicuous response has been shown to have an effect on the foraging and traffic by *Neodohrniphora curvinervis* (Malloch) on *Atta cephalotes* (L.) colonies in Costa Rica (Orr, 1992) as well as by *Neodohrniphora* spec. on *Atta sexdens* (L.) in the laboratory in Brazil (Bragança et al., 1998). To this date, only one study has been published that measured the effect of phorids in the field (Orr, 1992), but no study has evaluated the field impacts of leaf-cutting ant phorids at different parasitoid densities, which is of great relevance when designing augmentative biological control programs, because in general they include mass releases of the biological control agent. Although it has been shown that conspecific females of *Pseudacteon* spec. engage in competitive interference when attacking *Solenopsis invicta* Buren in laboratory conditions (Chirino et al., 2012), every published study with leaf-cutting ant phorids conducted in the laboratory has dealt with one phorid at a time (Bragança et al., 1998, 2008, 2009; Tonhasca et al., 2001; Gazal et al., 2007, 2009), and thus it is not possible to accurately predict what would happen in the field if several females attack on one trail.

The purpose of this study was to quantify the effect that *E. trilobata* exerts on *A. vollenweideri* colonies in the field at two different densities of phorids, to assess the impact that these parasitoids have on the food supply of the nest (as ant traffic and weight of ant load) and the type of food transported (as weight of monocotyledon and dicotyledon fragments).

Materials and methods

Study site and experimental unit

We conducted trials on *A. vollenweideri* colonies in the field at San Cristóbal, Santa Fe province, Argentina (30°12′S, 61°09′W), during spring (October–December 2010) and autumn (March–May 2011). Each trial was

performed on a foraging trail of a nest, which was carefully inspected for 1 h before the start of each attack to ensure that no phorids of any species were present on it. A second trail with no phorids was selected to act as control. Our field experience allowed us to select distant trails from the same nests. There is no evidence of communication between ants on such trails, at least not apparent in ant behavior; in fact, it has been proven that ants show high fidelity toward the same trail over time (Sousa-Souto et al., 2005; Elizalde & Farji-Brener, 2012).

Experiments

In the first sampling (T = 0), we collected every ant returning to the nest during a minute, as well as their loads, both from the attack trail and the control trail. After collecting this sample, we placed an isolation tunnel (3 m long, 40 cm wide, 30 cm high) over each trail to keep phorids away. We built tunnels of this length because numerous field observations (AC Guillade, unpubl.) indicated that *E. trilobata* attacks mainly on the 2 m of the foraging trail closest to the nest entrance, whereas *Myrmosicarius brandaoi* Disney, a sympatric species, focuses mainly on nest entrances. The walls of the tunnel were made of fine mesh cloth, and included several windows covered with transparent X-ray film, which allowed us to follow the activity of the flies on the trails. The structure was made of 0.5 mm thick wire, with ends long enough to be buried into the soil at the sides of the trail, so that the cloth walls touched the ground. Tunnels also included a small opening with a removable cap, through which flies could be introduced on the trail. After placing the tunnel on each trail, we released either one or four *E. trilobata* females on the attack trail. We chose these densities to represent the minimum possible (one phorid) and contrast it with the maximum parasitoid abundance per trail observed in our study site (L Elizalde, pers. comm.). Flies were collected the previous day with an aspirator and kept in plastic tubes with a cap adapted to contain a cotton plug soaked with a sugar water (40% wt/vol) solution. Thirty minutes after introducing the fly into the attack tunnel, a second sampling was conducted (T + 30), collecting every nest-bound ant and their loads during a minute, from both the attack trail and the control trail. Two subsequent samples were taken every 30 min (T + 60 and T + 90) from attack and control trails. After collecting the T + 90 sample, the flies were removed from the attack trail, and ants were allowed to forage for another 30 min, when the final sample (T + 120) was collected from both trails, thus ending the trial. Both control and attack trails were continually inspected to ensure that no flies were ever present on the control trails, and that only the fly or flies we had released were on the attack trails. If a fly in an attack tunnel escaped or was killed by ants, it was immediately replaced by a new female, so that phorid density in the attack tunnel remained constant throughout the assay. We performed 12 trials with a density of one phorid per trail, and 12 trials at a density of four phorids per trail, each paired with a control.

Ants collected from both control and attack trails were kept at room temperature in plastic containers with a plaster base, and fed sugar water (40% wt/vol) ad libitum. All ants from each sample were counted, to determine traffic at each sampling point, and measured under a Nikon SMZ800 (Tokyo, Japan) stereoscopic microscope with an ocular micrometer (width of the head capsule below the eyes), so as to assess whether the presence of flies had any effect on the distribution of size frequencies. We also determined the number of parasitized ants in each sample to assess whether parasitism rates were altered by the trials. Plant material carried by ants was sorted into monocotyledons and dicotyledons and dried to constant weight at 60 °C for 72 h before weighing. As we carried out our tests in spring and fall, the effect of phorid density (0, 1, or 4 females) on the type of plant material transported was assessed for both seasons.

Statistical analysis

We first conducted ANOVAs to compare T = 0 between control and treatments for traffic on the trails, weight of plant material transported, and size of ants. After ascertaining that there were no significant differences between control and treatments prior to the attacks, data for T + 30, T + 60, T + 90, and T + 120 were analyzed using repeated measures ANOVA, with Bonferroni's correction for multiple comparisons. Because data did not fulfill the assumption of sphericity, we reported a corrected F (F_C), using the Greenhouse-Geisser correction to adjust the degrees of freedom.

We performed an ANOVA to analyze parasitism rates across treatments. To assess the effect of our treatments on weight of monocotyledon and dicotyledon fragments carried, we performed non-parametric Kruskal–Wallis ANOVA, as data were not normally distributed, with Mann–Whitney tests for pair-wise comparisons, adjusting the value of α with the Bonferroni correction. Tests were carried out using STATISTIX (Analytical Software, 1998). All analyses were carried out using SYSTAT 13 (Systat Software, 2009).

Results

Ant traffic (Figure 1) did not differ between control (0 phorids) and attack trails (one and four phorids) at T = 0 ($F_{2,45} = 0.131$, P = 0.87). Repeated measures ANOVA

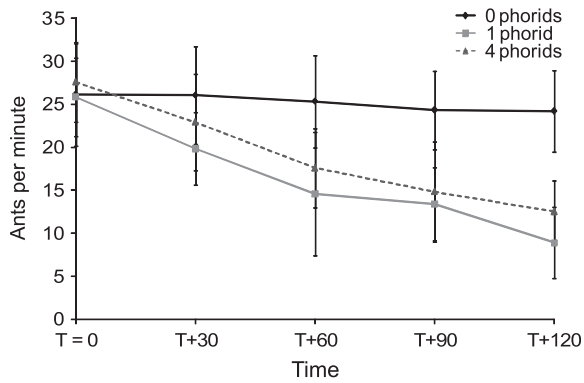**Figure 1** Traffic of *Atta vollenweideri* (mean ± SD no. ants/min) on foraging trails with 0, 1, or 4 phorids (*Eibesfeldtphora trilobata*) present, at 30-min intervals after release of the phorids (T = 0).
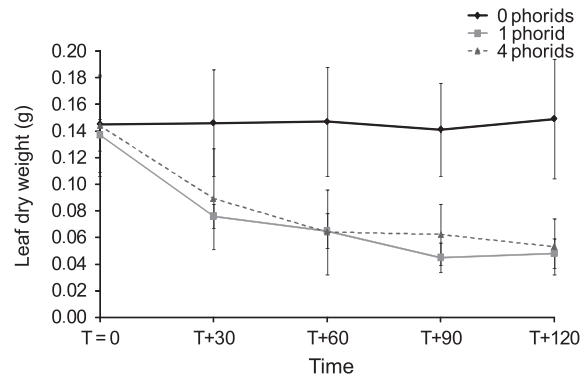

Figure 2 Mean (± SD) dry weight (g) of plant material carried on *Atta vollenweideri* foraging trails with 0, 1, or 4 phorids (*Eibesfeldtphora trilobata*) present, at 30-min intervals after release of the phorids (T = 0).

indicated that treatment had a significant effect on ant traffic ($F_{C2,45} = 54.11$, $P<0.0001$), across time ($F_{C2,45} = 18.35$, $P = 0.0005$), and the interaction between time and phorid density ($F_{C2,45} = 11.35$, $P<0.0001$). However, post-hoc comparisons exhibited no significant differences between attacks with one or four phorids ($P = 0.14$). Ant traffic decreased with both phorid densities tested, and continued to dwindle after the parasitoids were removed from the tunnels.

The amount of plant material transported into the colony was also affected by the presence of phorids (Figure 2). At T = 0, we found no significant differences between the material transported in the control trails which were opposite to attack trails ($F_{2,45} = 0.185$, $P = 0.83$). After this point, the introduction of female flies, at densities of either one or four per trail, resulted in reduction in plant material transported (treatment: $F_{C2,45} = 71.05$; time: $F_{2,45} = 17.42$; treatment*time: $F_{C2,45} = 22.74$, all $P<0.0001$). Again, there were no significant differences between the one- and four-phorid treatments ($P = 0.27$).

By sampling trails with no phorids in summer and winter to supplement the data from our attack assays, we found that, except in the fall, when monocotyledons amounted to 80.4% of the plant material transported, most of the weight of plant material carried into the nest on control trails throughout the four seasons corresponded to dicotyledons, whereas the proportion of monocotyledons ranged from 0.7% in summer to 25% in spring.

The presence of phorids altered the relative weight of monocotyledon and dicotyledon fragments entering the nest. During the fall trials (Figure 3A), the weight of monocotyledon fragments carried into the nest was
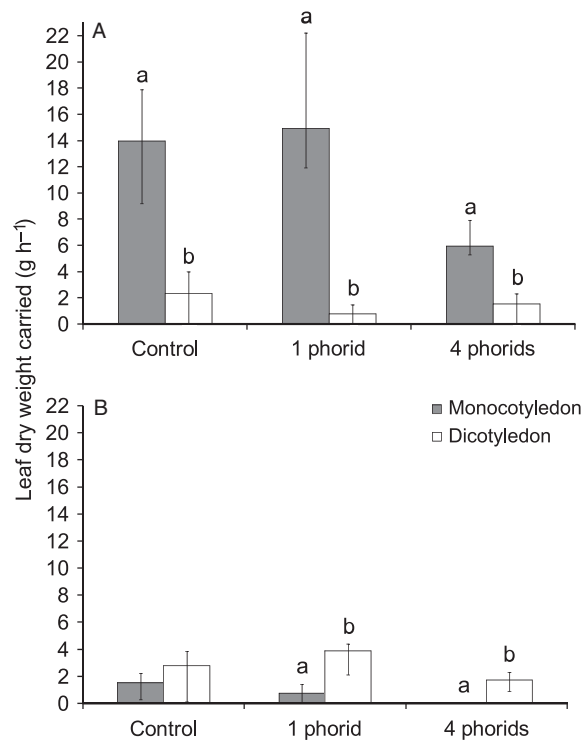

Figure 3 Median (+ first and third quartiles) dry weight (g) of monocotyledon and dicotyledon fragments carried per h into the *Atta vollenweideri* nest on trails with 0 (control), 1, or 4 phorids (*Eibesfeldtphora trilobata*) in (A) fall and (B) spring. Different letters within a treatment indicate significant differences.

significantly higher than the weight of dicotyledon fragments in all treatments (control: U = 36, P<0.0001; one phorid: U = 54, P = 0.0018; four phorids: U = 42, P = 0.0002). The weight of monocotyledons carried in

trails with four phorids was significantly lower than in control (U = 488, P = 0.0002) and one phorid (U = 426, P<0.0001) trails, whereas the weight of dicotyledon fragments was not significantly different across treatments (H = 0.59, P = 0.56). In spring (Figure 3B), the weight of dicotyledon fragments was higher than the weight of monocotyledons in trails with one phorid (U = 216) and four phorids (U = 697, both P<0.0001), whereas in control trails the difference was not significant (U = 764, P>0.05). The weight of dicotyledon fragments varied significantly across treatments (H = 9.14, P = 0.003), with Mann–Whitney comparisons showing significant differences between control trails and trails with four phorids (U = 809, P = 0.003) as well as between trails with one and four phorids (U = 426, P = 0.0002), but not between control and one-phorid trails (U = 367, P = 0.8). Monocotyledon fragments did not differ across treatments (H = 5.42, P>0.05).

The presence of phorids also had a remarkable effect on the frequency distribution of worker sizes in the ants returning to the nests (Figure 4). At T = 0 (Figure 4A), we found no differences between the size of workers in control trails (mean ± SD = 1.73 ± 0.35 mm) vs. those selected as to be later attacked with one phorid (1.74 ± 0.36 mm)

or four phorids (1.73 ± 0.28 mm) ($F_{2,817}$ = 0.58, P = 0.94). However, after introducing the fly or flies (Figure 4B–E), we found significant differences in the mean size of workers in phorid treatments relative to the control trails ($F_{2,817}$ = 18.23, P<0.0001), as well as through time ($F_{2,817}$ = 4.82, P = 0.0024) and the interaction between treatment and time ($F_{2,817}$ = 2.43, P = 0.023). As with traffic and plant material transported, we found no significant differences in the mean size of workers between the one- and four-phorid treatments (P>0.05).

Parasitism rates did not differ across treatments ($F_{2,45}$ = 0.08, P = 0.9).

Discussion

The presence of phorids on the foraging trails affected parameters of field colonies such as traffic of ants and amount of plant material transported into the nests, as well as the distribution of frequencies of worker ants on the trails. The weight of monocotyledons and dicotyledons carried into the nests was also affected by the presence of phorids at different densities. Traffic on attack trails was reduced to 50% of the traffic recorded on control trails by the end of the experiments. When one phorid alone was
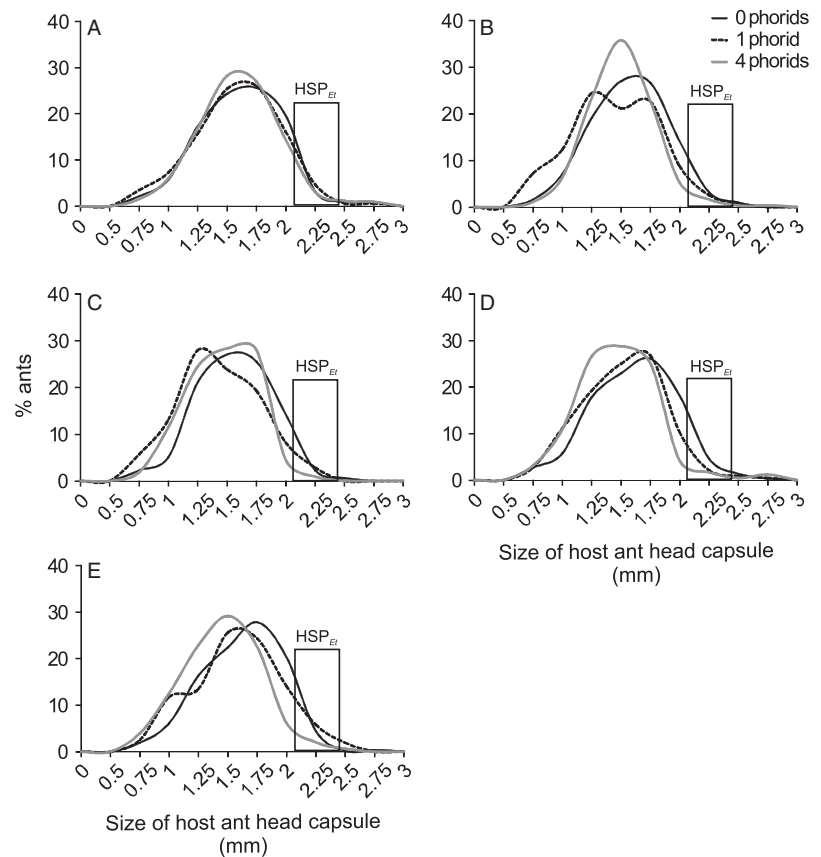

Figure 4 Frequency distribution of *Atta vollenweideri* sizes (head capsule width, mm) on experimental trails with 0, 1, or 4 phorids (*Eibesfeldtphora trilobata*), (A) before phorids were introduced into a priori assigned treatments, (B–D) after 30, 60, or 90 min, respectively, of phorids attacking, and (E) 30 min after removing phorids following the last sampling (90 min). HSP$_{Et}$: host size preferred by *E. trilobata* females for oviposition.

present, the reduction was ca. 20% greater than in trails with four phorids attacking simultaneously, although this difference was not statistically significant. The time phorids spent on the trails had an effect on traffic, with the reduction in ants per min increasing as time passed. We recorded a lag effect in which 30 min after removing the parasitoids from the trail, ant traffic continued to decrease. Such a lag effect has been reported previously for *Neodohrniphora* spec. on *A. sexdens* colonies under laboratory conditions (Bragança et al., 1998). These authors also found a reduction in traffic in the trails after releasing one phorid. Several factors can account for such a reduction. Although parasitism by phorids in general and by *E. trilobata* in particular can be rather low (Jouvenaz et al., 1981; Tonhasca, 1996; Bragança et al., 1998; Morrison, 1999; Guillade & Folgarait, 2011; but see Elizalde & Folgarait, 2011), ants in the presence of these parasitoids display strong reactions, which include freezing on the trail and adopting defensive postures (Elizalde & Folgarait, 2012). Workers recently attacked by *E. trilobata* have been observed to curl into themselves (Folgarait, 2013) and lay motionless for several minutes. These individual-level responses are likely influencing the foraging rhythm on the trails where phorids are present, as other ants in the trail (often smaller workers) form a circle around the attacked ant and touch it repeatedly with their antennae, a behavior termed by Elizalde & Folgarait (2012) as 'tending' (Bragança et al., 1998; Elizalde & Folgarait, 2012).

The amount of plant material carried into the nest was also affected by the presence of phorids on foraging trails. We observed a 66% reduction in dry weight carried into the nest by the end of the experiment, with the same lag effect we recorded in traffic. As with traffic, the slight difference between the one- and four-phorid treatments was not significant. The plant material transported cannot only be related to the traffic on the trails, but also to the size distribution of the workers foraging. *Atta vollenweideri* workers carry plant material into their colonies by forming transport chains (Röschard & Roces, 2002), with cutter ants cutting and dropping large grass fragments, which are subsequently cut into smaller pieces by smaller ants along the transport chain. Röschard & Roces (2003a) found that the size of the grass fragments chosen by foragers depended on the distance to the nest, with size-matching between ant size and fragment size being stronger as the distance from the nest decreased. Therefore, smaller ants (due to the effect of phorids) in the proximal end of the trail would result in smaller fragments taken into the colony, which would in turn affect performance (Roces & Bollazzi, 2009). For this to affect the whole colony in the field, *E. trilobata* females should, according to our results, be present on each active trail of the colony, with – at least

– one phorid per trail. The lack of significant differences in ant traffic and weight of plant material transported between trails attacked by either one or four phorids may be the result of intraspecific competition, as reported by Chirino et al. (2012) for *Pseudacteon tricuspis* Borgmeier females on *S. invicta*. However, the fact that in our experiment the parasitoids were forced to attack in a closed tunnel could bias our results, as in natural conditions females would be able to avoid interference by moving down the trail, away from other attacking females.

The frequency distribution of host sizes was altered by the presence of one or four phorids. Although there were no significant differences between the mean size of foragers on trails with one vs. four phorids, the distribution of size frequencies exhibited some discrepancies. In both cases, the number of workers in the preferred size for oviposition by *E. trilobata* (Elizalde & Folgarait, 2011; Guillade & Folgarait, 2011) became less frequent after the introduction of phorids, and the effect was more pronounced with four phorids on the trail, to the point that at T + 60 and T + 90, ants within this size range were 5× more abundant at the one-phorid trails than at the four-phorid trails. We observed a lag effect after removing the flies, which for host sizes was more pronounced when four phorids had been present, as at T + 120 the percentage of hosts within the preferred range was ca. 12% in control trails, ca. 10% in one phorid, and ca. 4% in four-phorid trails. Thus, the increase in phorid density prolonged the effect on host size distribution after the parasitoids had departed the trail.

Atta vollenweideri has been reported as a grass-cutter (Jonkman, 1976, 1980; Robinson & Fowler, 1982), and is considered as such ever since (Farji-Brener & Ruggiero, 1994; Röschard & Roces, 2003a,b, 2011). However, the results we obtained in our samplings lead us to propose that they should no longer be called grass-cutting ants, given that this is the first evidence that shows in detail the type of plant material carried by these ants through time. Interestingly, the weight of monocotyledons and dicotyledons carried was affected by the presence of phorids. In the fall, when the ants were carrying the greatest amount of plant material, the weight of monocotyledons carried decreased significantly in the presence of four phorids. Because of their shape, monocotyledon fragments might afford less protection to foragers and interfere with their running speed. *Atta vollenweideri* is known to accumulate piles of plant fragments on the trail at irregular intervals, forming transport chains to carry them into the colony (Röschard & Roces, 2003b). The presence of phorids on the trail is likely to demand greater transport speed to relay the information regarding the threat of parasitism (Röschard & Roces, 2003b), and in this context it would be advantageous for the foragers to select more

dicotyledons from these cache piles, which, because of their shape, allow them greater speed and better protection against phorids. This effect could also be observed in spring, when in control trails there were no significant differences between weight of monocotyledons and dicotyledons carried, whereas in trails with one and four phorids the weight of dicotyledons carried was significantly greater than the weight of monocotyledons.

Also interesting was the swiftness of the response of the colony to the appearance of phorids on a trail. At a density of one or four females per trail, the colony responded with a re-distribution of the size of foragers on the trail, which was evident in the first sampling after introducing the phorids. Elizalde & Folgarait (2012) reported that 17% of the ants in the trail run back into the nest upon encountering a phorid, whereas others drop their loads when adopting defensive postures. Ants returning to the nest are likely relaying the information that parasitoids have arrived on the trail, and this information can then trigger a colony-level response in the form of reducing the proportion of majors and increasing the proportion of smaller ants on the trail. This response was limited to the trail under attack, as we found no evidence of changes in size distribution in control trails. This seems to support the hypothesis advanced by Gordon (1999, 2007), who proposed that colony-level organization is dependent on the workers rather than the queen, and that the interactions between workers result in changes in task allocation. Our results show that the response was limited to those trails affected by attacking phorids, whereas on unaffected trails foraging continued without changes of forager size, traffic, and plant material transported.

Parasitism by *E. trilobata* did not differ significantly between control and treatments (at either density of females), but this is not completely unexpected. Our experimental design is likely influencing the results, as the tunnels prevented us from collecting the attacked ants specifically. Besides, the fact that parasitized ants can be collected from foraging trails of different treatments in similar proportions (Erthal & Tonhasca, 2000; Elizalde & Folgarait, 2011; Guillade & Folgarait, 2011) suggests that these workers remain active for several days after being parasitized, but there are no data on what happens with a worker immediately after attack. We have often observed minimal workers tending to an attacked forager, which lies immobile for several seconds before returning to the nest. It is possible that, once inside the nest, this ant spends some time relaying information on the presence of parasitoids. Even if it immediately returns to its task of carrying plant fragments, it takes workers a few hours to complete the trip to the cutting site and back, along trails that often exceed 100 m in length (Röschard & Roces, 2011). Thus, it is highly unlikely that we could capture a recently parasitized ant in our random sampling. Nonetheless, the importance of behavioral effects of ants triggered by phorids, relative to direct mortality by parasitism, has been reported abundantly (Feener & Brown, 1992; Orr, 1992; Folgarait & Gilbert, 1999; Morrison, 1999; Feener, 2000; LeBrun & Feener, 2007; Feener et al., 2008).

Our results are relevant from an applied point of view. We have quantified the response of field colonies to attacking phorids and shown that, even at the minimum possible density, these parasitoids can exert a significant effect on a key aspect of colony life, the consumption of food through foraging. We carried out our experiments with only one phorid species, but previous work on Argentine phorids (Elizalde & Folgarait, 2011, 2012; Guillade & Folgarait, 2011) demonstrates that different species of phorids attacking the same host partition this resource through several complementary strategies. *Myrmosicarius brandaoi* prefers smaller workers than *E. trilobata* (1.59–2.01 mm head width), and *Myrmosicarius gonzalezae* Disney attacks ants between 1.51 and 1.9 mm; both search for their hosts at nest entrances more often than at foraging trails (Elizalde & Folgarait, 2011; Guillade & Folgarait, 2011). Therefore, even when worker ants in the size range preferred by *E. trilobata* become scarcer when this species is attacking, other phorid species can exploit smaller workers. It would be interesting to evaluate phorid attacks using parasitoids that prefer different sizes of workers, to determine the effect that two or more complementary species of phorids may have on the frequency distribution of worker sizes on the trails. Although ant colonies have evolved several defense mechanisms against phorid flies, such as switching to nocturnal foraging, as demonstrated by Orr (1992), *M. brandaoi* has been collected from foraging trails after dusk (Disney et al., 2006), which suggests that, in true arms race form, parasitoids are evolving to compensate for diel shifts in foraging by ants. *Apocephalus setitarsus* Brown, on the other hand, is likely to specialize on large hosts (Elizalde & Folgarait, 2011), and it attacks ants at cutting sites. Furthermore, seasonal abundance varies between species (Elizalde & Folgarait, 2010, 2011; Guillade & Folgarait, 2011), which would help competing species to partition their host resource temporally as well as spatially. Therefore, these three species could act together as control agents avoiding interspecific competition, covering a wider range of host sizes. Future work should test this hypothesis in the field.

Acknowledgements

References

Analytical Software (1998) Statistix for Windows User's Manual. Analytical Software, Tallahassee, FL, USA.

Boaretto MAC & Forti LC (1997) Perspectivas no controle de formigas cortadeiras. Serie Técnica IPEF 11: 31–46.

Bragança MAL, Tonhasca A Jr & Della Lucia TMC (1998) Reduction in the foraging activity of the leaf-cutting ant *Atta sexdens* caused by the phorid *Neodohrniphora* sp. Entomologia Experimentalis et Applicata 89: 305–311.

Bragança MAL, Machado de Souza L, Nogueira CA & Della Lucia TMC (2008) Parasitismo por *Neodohrniphora* spp. Malloch (Diptera: Phoridae) em operarias de *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). Revista Brasileira de Entomologia 52: 300–302.

Bragança MAL, Tonhasca A Jr & Della Lucia TMC (2009) Características biológicas e comportamentais de *Neodohrniphora elongata* Brown (Diptera: Phoridae), um parasitoide da saúva *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). Revista Brasileira de Entomologia 53: 600–606.

Callcott AA, Porter SD, Weeks RD Jr, Graham LC, Johnson SJ & Gilbert LE (2011) Fire ant decapitating fly cooperative release programs (1994-2008): two *Pseudacteon* species (*P. tricuspis* and *P. curvatus*) rapidly expand across imported fire ant populations in the southeastern United States. Journal of Insect Science 11: 19.

Cherret JM (1986) The economic importance and control of leaf-cutting ants. Economic Impact and Control of Social Insects (ed. by SB Vinson), pp. 165–192. Praeger Publishers, New York, NY, USA.

Chirino MG, Folgarait PJ & Gilbert LE (2012) *Pseudacteon tricuspis*: its behavior and development according to the social form of its host and the role of interference competition among females. Journal of Economic Entomology 105: 386–394.

De Coll OR (1998) Ensayo de Control de *Acromyrmex lobicornis* Emery (Formicidae) con Cebo Fluramim (Sulfluramida). INTA, Montecarlo, Argentina.

Disney RHL (1994) Scuttle Flies: The Phoridae. Chapman and Hall, London, UK.

Disney RHL, Elizalde L & Folgarait PJ (2006) New species and revision of *Myrmosicarius* (Diptera: Phoridae) that parasitize leaf-cutter ants (Hymenoptera: Formicidae). Sociobiology 47: 771–809.

Disney RHL, Elizalde L & Folgarait PJ (2009) New species and records of scuttle flies (Diptera: Phoridae) that parasitize leaf-cutter and army ants (Hymenoptera: Formicidae). Sociobiology 54: 601–632.

Elizalde L & Farji-Brener AG (2012) To be or not to be faithful: flexible fidelity to foraging trails in the leaf-cutting ant *Acromyrmex lobicornis*. Ecological Entomology 37: 370–376.

Elizalde L & Folgarait PJ (2010) Host diversity and environmental variables as determinants of leafcutter phorid parasitoid's species richness. Journal of Biogeography 37: 2305–2316.

Elizalde L & Folgarait PJ (2011) Biological attributes of Argentinian phorid parasitoids (Insecta: Diptera: Phoridae) of leaf-cutting ants, *Acromyrmex* and *Atta*. Journal of Natural History 45: 2701–2723.

Elizalde L & Folgarait PJ (2012) Behavioral strategies of phorid parasitoids and responses of their hosts, the leafcutter ants. Journal of Insect Science 12: 135.

Erthal M Jr & Tonhasca A Jr (2000) Biology and oviposition behavior of the phorid *Apocephalus attophilus* and the response of its host, the leaf-cutting ant *Atta laevigata*. Entomologia Experimentalis et Applicata 95: 71–75.

Farji-Brener AG & Ruggiero A (1994) Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range size. Journal of Biogeography 21: 391–399.

Feener DH Jr (1981) Competition between ant species: outcome controlled by parasitic flies. Science 241: 815–817.

Feener DH Jr (2000) Is the assembly of ant communities mediated by parasitoids? Oikos 90: 79–88.

Feener DH Jr & Brown BV (1992) Reduced foraging of *Solenopsis geminata* (Hymenoptera: Formicidae) in the presence of parasitic *Pseudacteon* spp. (Diptera: Phoridae). Annals of the Entomological Society of America 85: 80–84.

Feener DH Jr & Moss KAG (1990) Defense against parasites by hitchhikers in leaf-cutting ants: a quantitative assessment. Behavioral Ecology and Sociobiology 26: 17–29.

Feener DH Jr, Orr MR, Wackford KM, Longo JM, Benson WW & Gilbert LE (2008) Geographic variation in resource dominance-discovery in Brazilian ant communities. Ecology 89: 1824–1836.

Filho OP & Dorval A (2003) Efeito de formulações granuladas de diferentes produtos químicos e à base de folhas e de sementes de gergelim, *Sesamum indicum*, no controle de formigueiros de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae). Ciência Florestal 13: 67–70.

Foguelman D (2003) Plagas y Enfermedades en Manejo Orgánico: Una Mirada Latinoamericana. IFOAM, Bonn, Germany.

Folgarait PJ (2013) Leaf-cutter ants' parasitoids: current knowledge. Psyche 2013: e539780.

Folgarait PJ & Gilbert LE (1999) Phorid parasitoids affect foraging activity of *Solenopsis richteri* under different availability of food in Argentina. Ecological Entomology 24: 163–173.

Forest Stewardship Council (2007) FSC guidance document. FSC Pesticides policy: guidance on implementation. Available at: http://ebookbrowse.com/fsc-pol-30-001-en-fsc-pesticides-policy-2005-pdf-d342630382 (accessed on 5 May 2014).

Forest Stewardship Council (2012) FSC pesticides policy guidance addendum: list of approved derogations for use of 'highly

hazardous' pesticides. Available at: http://www.fsc.org.download.fsc-gui-30-001a-v1-0-en.113.htm (accessed on 5 May 2014).

Gazal VS, Bailez O, Bailez AM & Tonhasca A Jr (2007) Effect of the size of workers of *Atta sexdens rubropilosa* on the attack behavior of *Neodohrniphora* spp. (Diptera: Phoridae). Sociobiology 50: 35–44.

Gazal VS, Bailez O & Bailez AM (2009) Mechanism of host recognition in *Neodohrniphora elongata* (Brown) (Diptera: Phoridae). Animal Behaviour 78: 1177–1182.

Gordon DM (1999) Interaction patterns and task allocation in ant colonies. Information Processing in Social Insects (ed. by C Detrain, JL Deneubourg & JM Pasteels), pp. 51–67. Birkhäuser, Basel, Switzerland.

Gordon DM (2007) Control without hierarchy. Nature 446: 143.

Guillade AC & Folgarait PJ (2011) Life history traits and parasitism rates of four phorid species (Diptera: Phoridae), parasitoids of *Atta vollenweideri* (Hymenoptera: Formicidae) in Argentina. Journal of Economic Entomology 104: 32–40.

Guillade AC & Folgarait PJ (2014) Natural enemies of *Atta vollenweideri* (Hymenoptera: Formicidae) leaf-cutter ants negatively affected by synthetic pesticides, chlorpyrifos and fipronil. Journal of Economic Entomology 107: 105–114.

Hölldobler B & Wilson EO (1990) The Ants. Springer, Berlin, Germany.

Jonkman JCM (1976) Biology and ecology of the leaf cutting ant *Atta vollenweideri* Forel, 1893. Zeitschrift für Angewandte Entomologie 81: 140–148.

Jonkman JCM (1980) Average vegetative requirements, colony size and estimated impact of *Atta vollenweideri* on cattle-raising in Paraguay. Zeitschrift für Angewandte Entomologie 89: 135–143.

Jouvenaz DP, Lofgren CS & Banks WA (1981) Biological control of imported fire ants: a review of current knowledge. Entomological Society of America Bulletin 27: 203–208.

LeBrun EG (2005) Who is the top dog in ant communities? Resources, parasitoids and competitive hierarchies. Oecologia 142: 643–652.

LeBrun EG & Feener DH Jr (2007) When trade-offs interact: balance of terror enforces dominance discovery trade-off in a local ant assemblage. Journal of Animal Ecology 76: 58–64.

Morrison LW (1999) Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. Oecologia 121: 113–122.

Morrison LW & Porter SD (2005) Phenology and parasitism rates in introduced populations of *Pseudacteon tricuspis*, a parasitoid of *Solenopsis invicta*. BioControl 50: 127–141.

Orr MR (1992) Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 30: 395–402.

Philpott SM (2005) Trait-mediated effects of parasitic phorid flies (Diptera: Phoridae) on ant (Hymenoptera: Formicidae) competition and resource access in coffee agro-ecosystems. Environmental Entomology 34: 1089–1094.

Plowes RM, LeBrun EG & Gilbert LE (2011) Introduction of the fire ant decapitating fly *Pseudacteon obtusus* in the United States: factors influencing establishment in Texas. BioControl 56: 295–304.

Plowes RM, Folgarait PJ & Gilbert LE (2012) The introduction of the fire ant parasitoid *Pseudacteon nocens* in North America: challenges when establishing small populations. BioControl 57: 503–514.

Porter SD, Graham LC, Johnson SJ, Thead LG & Briano JA (2011) The large decapitating fly *Pseudacteon litoralis* (Diptera: Phoridae): successfully established on fire ant populations in Alabama. Florida Entomologist 94: 208–213.

Robinson SW & Fowler HG (1982) Foraging and pest potential of Paraguayan grass-cutting ants (*Atta* and *Acromyrmex*) to the cattle industry. Zeitschrift für Angewandte Entomologie 93: 42–54.

Roces F & Bollazzi M (2009) Information transfer and the organization of foraging in grass- and leaf-cutting ants. Food Exploitation by Social Insects: Ecological, Behavioral, and Theoretical Approaches (ed. by S Jarau & M Hrncir), pp. 261–275. CRC Press, Boca Raton, FL, USA.

Röschard J & Roces F (2002) The effect of load length, width and mass on transport rate in the grass-cutting ant *Atta vollenweideri*. Oecologia 131: 319–324.

Röschard J & Roces F (2003a) Fragment-size determination and size-matching in the grass-cutting ant *Atta vollenweideri* depend on the distance from the nest. Journal of Tropical Ecology 19: 647–653.

Röschard J & Roces F (2003b) Cutters, carriers and transport chains: distance-dependent foraging strategies in the grass-cutting ant *Atta vollenweideri*. Insectes Sociaux 50: 237–244.

Röschard J & Roces F (2011) Sequential load transport in grass-cutting ants (*Atta vollenweideri*): maximization of plant delivery rate or improved information transfer? Psyche 2011: e643127.

Sousa-Souto L, Hay JD, Ambrogi BG & Kitayama K (2005) Trail fidelity and its influence on foraging by *Acromyrmex niger* (Hymenoptera: Formicidae). Sociobiology 45: 129–136.

Systat Software (2009) SYSTAT 13 for Windows User's Manual. Systat Software, Chicago, IL, USA.

Tonhasca A Jr (1996) Interactions between a parasitic fly, *Neodhorniphora declinata* (Diptera: Phoridae) and its host, the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Ecotropica 2: 157–164.

Tonhasca A Jr, Bragança MAL & Erthal M Jr (2001) Parasitism and biology of *Myrmosicarius grandicornis* (Diptera: Phoridae) in relationship to its host, the leaf-cutting ant *Atta sexdens* (Hymenoptera: Formicidae). Insectes Sociaux 48: 154–158.

Weber NA (1972) Gardening Ants, The Attines. American Philosophical Society, Philadelphia, PA, USA.

Zanetti R, Zanuncio JC, Mayhé-Nunes AJ, Barros Medeiros AG & Souza-Silva A (2003) Combate sistemático de formigas cortadeiras com iscas granuladas, em eucaliptais com cultivo mínimo. Revista Árvore 27: 387–932.