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Source: Florida Entomologist, 103(1) : 112-115

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.103.0418>

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# Seasonal parasitism of the leaf-cutting ant *Atta sexdens* Linnaeus (Hymenoptera: Formicidae) by phorid flies (Diptera: Phoridae) in a Brazilian Cerrado-Atlantic Forest ecotone

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## Abstract

Phorid flies (Diptera: Phoridae) parasitize insects of many orders including leaf-cutting ants, which are pests of several crops. These parasitoids may have a significant impact on ant behavior by altering their foraging rhythm. Seasonal variations in climatic variables, such as temperature and humidity, influence the abundance, activity period, distribution, and reproduction of insects. Therefore, knowledge of parasitoid seasonal patterns is important for biological control success. In this study we evaluate the parasitism of the leaf-cutting ant *Atta sexdens* L. (Hymenoptera: Formicidae) by *Apocephalus attophilus* Borgmeier and *Eibesfeldtphora tonhascai* Brown (both Diptera: Phoridae) across seasons throughout a yr in a Brazilian Cerrado-Atlantic Forest ecotone, and compare their parasitism in a semi-deciduous seasonal forest fragment and urban area. The number of ants parasitized by *A. attophilus* and *E. tonhascai* was higher in the winter and spring, respectively, but similar in summer and fall. *Apocephalus attophilus* parasitism was negatively correlated with temperature but not with relative humidity, whereas ant parasitism by *E. tonhascai* presented no correlation with either parameter. The number of ants parasitized by *A. attophilus* and *E. tonhascai* in the semi-deciduous seasonal forest fragment was higher than in urban areas. Knowledge of biotic and abiotic factors influencing the population dynamics of phorid flies is important for rearing and releasing protocols for these parasitoids.

Key Words: parasitoid; pests; temperature; humidity; urban area

## Resumo

Moscas forídeas (Diptera: Phoridae) parasitam insetos de várias ordens, incluindo formigas cortadeiras que são pragas de várias culturas. Esses parasitoides podem ter um impacto significativo no comportamento das formigas, alterando seu ritmo de forrageamento. Variações sazonais nas variáveis climáticas, como temperatura e umidade, influenciam a abundância, período de atividade, distribuição e reprodução dos insetos. Portanto, o conhecimento dos padrões sazonais dos parasitoides é importante para o sucesso do controle biológico. Aqui, nós avaliamos o parasitismo da formiga cortadeira *Atta sexdens* L. (Hymenoptera: Formicidae) por *Apocephalus attophilus* e *Eibesfeldtphora tonhascai* (ambos Diptera: Phoridae) ao longo das estações durante um ano em um ecótono Cerrado-Mata Atlântica, e comparamos seu parasitismo em um fragmento de floresta estacional semidecidual e área urbana. O número de formigas parasitadas por *A. attophilus* e *E. tonhascai* foi maior no inverno e na primavera, respectivamente, mas semelhante no verão e outono. O número de formigas parasitadas por *A. attophilus* foi negativamente correlacionado com a temperatura, mas não com a umidade relativa, enquanto o parasitismo por *E. tonhascai* não apresentou correlação com nenhum dos parâmetros. O número de formigas parasitadas por *A. attophilus* e *E. tonhascai* no fragmento de floresta estacional semidecidual foi maior que nas áreas urbanas. O conhecimento de fatores bióticos e abióticos que influenciam a dinâmica populacional de forídeos é importante para a elaboração de protocolos de criação e liberação desses parasitoides.

Palavras Chave: parasitoide; pragas; temperatura; umidade; área urbana

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Phorid flies are small-sized, highly diverse insects, found in Neotropical regions with varying larval lifestyles (Disney 1994). Many species of this family parasitize bees (Core et al. 2012), termites (Neoh & Lee 2010), and leaf-cutting ant pests of several crops (Della Lucia et al. 2014; Farder-Gomes et al. 2016). Leaf-cutting ants are managed principally with insecticidal baits (Zanetti et al. 2003; Montoya-Lerma et al. 2012). Increasing global awareness regarding sustainable cultivation and pest control methods that are safer for the environment (Bale et al. 2008; Zheng et al. 2017) has driven the search for alternative methods to manage leaf-cutting ants.

Leaf-cutting ant parasitoids in the family Phoridae are specialists or generalists, coexisting in the environment and attacking ants of different head sizes (Bragança & Medeiros 2006). Additionally, these parasitoids can reduce plant material transported into the nests and ant traffic on trails (Martinez et al. 2014; Guillade & Folgarait 2015). Females of these insects lay an egg into the body of each ant with the larva subsequently feeding on host tissues (Bragança & Medeiros 2006; Farder-Gomes et al. 2016). Phorid flies have an evolutionary relationship with host ants, and require them as the only food source for their entire development. Therefore, fly richness correlates with ant richness (Elizalde & Folgarait 2010).

Landscape structure is important for parasitoid-host interaction (Thies et al. 2005; Liu et al. 2016b), and fragmentation and loss of environmental complexity may decrease natural enemy abundance (Elizalde & Queiroz 2013; Zhao et al. 2015). Highly diversified landscapes may provide multiple resources, such as nest sites, temporary refuges, and food (Alignier et al. 2014; Liu et al. 2016b), whereas agricultural landscapes, e.g., pasture and field crops, have fewer resources due to reduced habitat complexity (Kleijn et al. 2009; Thies et al. 2011).

Changes in landscape structure also modifies microclimate, thereby affecting insect populations (Alignier et al. 2014; Aranda & Graciolli 2015). Climatic variables, such as temperature and humidity, influence the abundance, activity period, diversity, distribution, and reproduction of insects (Folgarait et al. 2005, 2007; Aranda & Graciolli 2015). Preferred climatic conditions differ between parasitoid species (Bragança & Medeiros 2006; Reese & Philpott 2012). For example, *Pseudacteon laciniosus* Brown (Diptera: Phoridae) was found to be more abundant in the wet season than in the dry, whereas *Pseudacteon planidorsalis* Brown (Diptera: Phoridae) showed the opposite pattern (Reese & Philpott 2012). Therefore, knowledge of parasitoid seasonal patterns is important for success of biological control. The objective of our study was to evaluate the parasitism of the leaf-cutting ant *Atta sexdens* Linnaeus (Hymenoptera: Formicidae) by the phorids *Apocephalus attophilus* Borgmeier and *Eibesfeldtphora tonhascai* Brown (both Diptera: Phoridae) across seasons throughout a yr in a Brazilian Cerrado-Atlantic Forest ecotone, and compare their ant parasitism in a semi-deciduous seasonal forest fragment and urban area.

## Materials and Methods

### STUDY SITE

This study was conducted at the Universidade Federal de Viçosa campus Florestal (19.871194°S, 44.423916°W) in Florestal, Minas Gerais State, Brazil. This region corresponds to the Cerrado-Atlantic Forest ecotone (IBGE 2018). Mean temperature and relative humidity of each mo were obtained through the weather station on campus. The seasons are as follows: spring (Sep–Nov), summer (Dec–Feb), fall (Mar–May), and winter (Jun–Aug).

### COLLECTION AND ANT REARING

Four *A. sexdens* nests were selected: 2 nests in a semi-deciduous seasonal forest fragment: (Nest 1: 19.876777°S, 44.413638°W) and (Nest 2: 19.884750°S, 44.416388°W); and 2 in urban areas around the campus: (Nest 3: 19.883750°S, 44.424055°W) and (Nest 4: 19.884833°S, 44.426611°W). The semi-deciduous seasonal forest fragment has an area of 7.1 ha, with the canopy not exceeding 20 m in height and with signs of anthropic disturbances. The ant nests within the forest fragment and urban area were isolated from each other by 200 and 300 m, respectively.

Approximately 200 workers were collected from each nest per mo between Nov 2012 and Nov 2013. Ants were collected between 7:00 PM and 10:00 PM along foraging trails. If ants were not foraging, a stick of wood was introduced into the nest entrance, and as soon as the ants left the nest, they were collected using forceps.

In the laboratory, ants of the same cohort nest were kept in a common plastic tray, fed daily with 10% honey water solution, and kept in an acclimatized chamber (25 ± 1 °C, 80 ± 5% RH, and 12:12 h [L:D] photoperiod). Dead ants were removed each d from trays and individualized in glass tubes sealed with cotton wool. These glass tubes were maintained in an acclimatized chamber and after 3 d, dead ants were observed under stereomicroscope (Zeiss Stemi DV4, Zeiss, Göttingen, Germany), to evaluate if they were parasitized by phorids. Parasitized ants were kept in the chamber until adult phorid emergence, and ants that were still alive 15 d after collection were discarded (Farder-Gomes et al. 2016). Flies were stored in plastic microtubes with 70% alcohol for further identification. Voucher specimens were deposited at the Laboratory of Entomology and Myrmecology, Universidade Federal de Viçosa campus Florestal, Florestal, Minas Gerais State, Brazil.

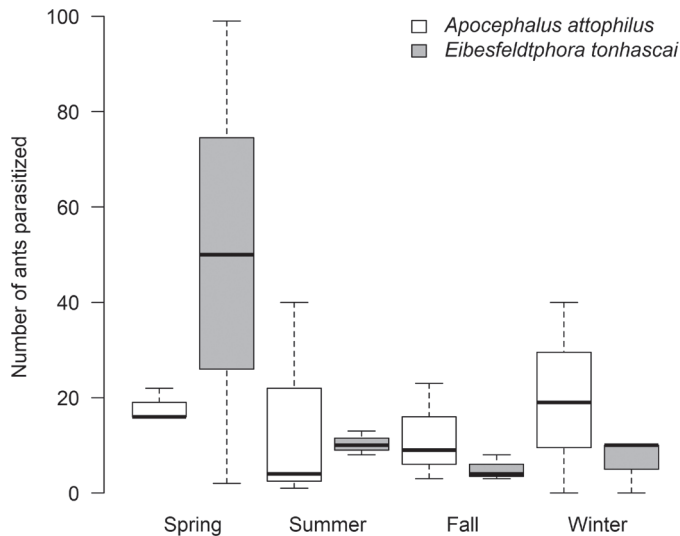
### STATISTICAL ANALYSES

A chi-square test of independence was performed to determine if there was a significant relationship between the level of phorid parasitism and seasons, as well as the abundance of leaf-cutting ants parasitized by *A. attophilus* and *E. tonhascai* during seasons. Pearson's Correlation Coefficient was used to assess the relationship between the number of ants parasitized by either species to temperature and relative humidity. Moreover, the number of ants parasitized by *A. attophilus* and *E. tonhascai* in semi-deciduous seasonal forest and urban landscape was compared using chi-square test. All analyses were performed using R Statistical Software (R Core Team 2018), and differences were considered significant when  $P < 0.05$ .

## Results

Parasitism of ants by phorid species was significantly influenced by season ( $\chi^2 = 74.6$ ; df = 3;  $P < 0.05$ ). The number of ants parasitized by *A. attophilus* was higher in the winter ( $\chi^2 = 33.3$ ; df = 1;  $P < 0.05$ ), and those by *E. tonhascai* in the spring ( $\chi^2 = 27.6$ ; df = 1;  $P < 0.05$ ) (Fig. 1). However, the number of ants parasitized by both parasitoids in summer ( $\chi^2 = 0.7$ ; df = 1;  $P > 0.05$ ) and fall ( $\chi^2 = 7.22$ ; df = 1;  $P > 0.05$ ) was similar (Fig. 1).

The number of ants parasitized by *A. attophilus* was negatively correlated with temperature ( $r = -0.722$ ; df = 9;  $P < 0.05$ ), but not with relative humidity ( $r = 0.150$ ; df = 9;  $P > 0.05$ ) (Fig. 2). The number of ants parasitized by *E. tonhascai* did not correlate with temperature ( $r = 0.040$ ; df = 9;  $P > 0.05$ ) or relative humidity ( $r = 0.243$ ; df = 9;  $P > 0.05$ ). In addition, parasitism by *A. attophilus* and *E. tonhascai* was significantly greater in the semi-deciduous (364 ants parasitized) ( $\chi^2 = 234.4$ ; df = 1;  $P < 0.05$ ) than the urban landscape (32 ants parasitized).



**Fig. 1.** Number of leaf-cutting ants *Atta sexdens* parasitized by *Apocephalus attophilus* and *Eibesfeldtphora tonhascai* in a Brazilian Cerrado-Atlantic Forest ecotone. The seasons are as follows: spring (Sep–Nov), summer (Dec–Feb), fall (Mar–May), and winter (Jun–Aug).

## Discussion

We found that an increase in number of leaf-cutting ants parasitized by *A. attophilus* occurred with decreasing temperature, whereas the highest parasitism by this species appeared during winter, which indicated this species has a greater tolerance to low temperatures, similar to that reported for the phorids *Pseudacteon borgmeieri* Schmitz (Diptera: Phoridae) (Folgarait et al. 2003) and *Lucianaphora folgaraitae* Disney (Diptera: Phoridae) (Elizalde et al. 2018). A negative correlation between parasitism by *A. attophilus* and temperature also was reported from Viçosa, Minas Gerais, Brazil (Martins 2015). Phorid flies commonly disappear during colder months (Folgarait et al. 2003). Thus, a preference for lower temperatures may benefit *A. attophilus* by allowing it to explore hosts during periods where few parasitoids of other species may be present. In addition, results of our work showed that parasitism rates of *A. attophilus* on *A. sexdens* were not correlated with the relative humidity; conversely, other studies have reported an increase in parasitism by

this species in the hottest and rainy seasons (Arruda et al. 2018; Fardner-Gomes et al. 2018). This suggests that *A. attophilus* possesses a wide range of tolerance for some environmental conditions. On the other hand, greater leaf-cutter ant parasitism during the spring by *E. tonhascai* suggested better adaptation of this species to the climatic conditions of that season. Differences in the number of ants parasitized between seasons may allow for temporal segregation of parasitoid species throughout the year with reduced interspecific competition (Guillade & Folgarait 2011; Arruda et al. 2018; Elizalde et al. 2018).

As in our study, lack of correlation between *E. tonhascai* parasitism with either temperature or humidity may indicate that this parasitoid is more resistant to changes in climatic conditions. *Apocephalus attophilus* can parasitize leaf-cutting ants during the day and night, but *E. tonhascai* is active only during the day (Bragança et al. 2008). We hypothesize that light intensity may be an important determinant of abundance and parasitism, similar to that reported for *Pseudacteon litoralis* Borgmeier (Diptera: Phoridae) attacking *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) in Rio Claro, São Paulo, Brazil (Pesquero et al. 1996), but this hypothesis would need further investigation. The highest number of ants parasitized in the semi-deciduous forest fragment may be due to the landscape diversity of this area with alternative sites for feeding and refuge, enhancing parasitoid populations (Pozzebon et al. 2015; Liu et al. 2016b; Damien et al. 2017). This agrees with the higher number of ants parasitized by *E. tonhascai*, *Eibesfeldtphora bragancai* Brown (Diptera: Phoridae), and *A. attophilus* in a Cerrado fragment containing *Eucalyptus* sp. (Myrtaceae), showing that these parasitoids are sensitive to changes in landscape composition (Pesquero et al. 2010). Similarly, high landscape diversity increased the *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) parasitism rates on *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) eggs (Liu et al. 2016a). The failure of the parasitoid *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae) to establish itself in the states of Florida and Tennessee, USA, was attributed to insufficient vegetation cover and harsh environmental conditions (Graham et al. 2003). Therefore, the maintenance of native vegetation fragments is important to enhance natural enemy populations.

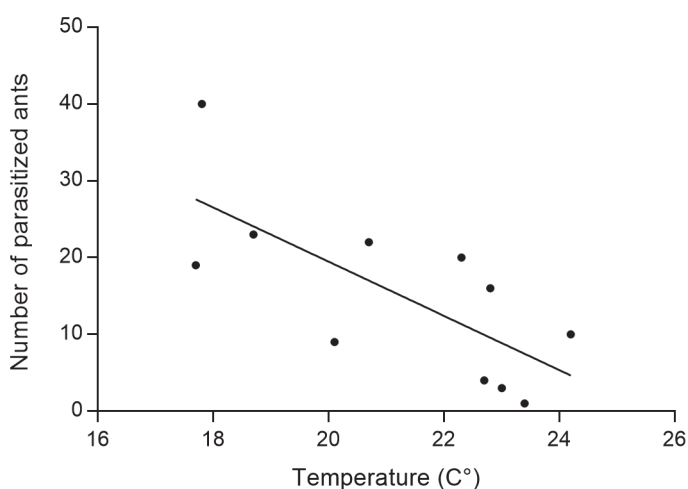
In summary, our study provides important ecological information about the interaction between the leaf-cutting ant *A. sexdens* and its parasitoids. Generally, the parasitism by phorid flies varied with season; *A. attophilus* parasitized more ants in winter and *E. tonhascai* in spring. Parasitism by *A. attophilus* was negatively correlated with temperature, and corroborates the preference of this species for lower temperatures. In addition, reduction of landscape complexity in urban areas may decrease the parasitism by parasitoid species. Therefore, biotic and abiotic factors that influence the population dynamics of phorids in the field must be understood to design mass rearing and releasing protocols for these parasitoids.

## Acknowledgments

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – (Finance Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG and Programa Cooperativo sobre Proteção Florestal (PROTEF) do Instituto de Pesquisas e Estudos Florestais (IPEF)). Phillip John Villani (University of Melbourne, Australia) corrected an edited English version of the manuscript.

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**Fig. 2.** Correlation between temperature and the number of leaf-cutting ants *Atta sexdens* parasitized by *Apocephalus attophilus* ( $r = -0.722$ ;  $df = 9$ ;  $P < 0.05$ ).



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