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Analysis of *Tiphia* parasitoids preovipositional behaviors and of their scarab host defensive responses

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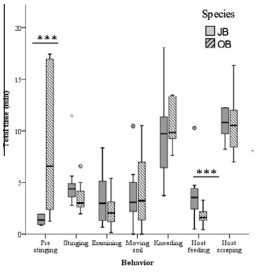
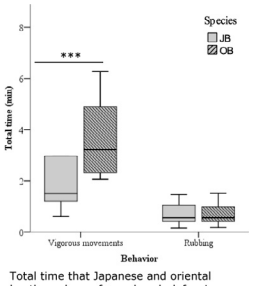
HIGHLIGHTS

- We examine how tiphiid wasps handle Japanese and oriental beetle grubs.
- We examine the defensive behaviors of scarab grubs.
- *Tiphia vernalis* spent longer time trying to sting oriental beetles.
- Oriental beetles spent longer time on defensive behaviors.
- Oriental beetle grubs are less susceptible to *T. vernalis* attack.

GRAPHICAL ABSTRACT



Total time that *Tiphia vernalis* performed each preovipositional behavior



Total time that Japanese and oriental beetle grubs performed each defensive behavior before being stung by *T. vernalis*.

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ABSTRACT

Tiphia vernalis Rohwer and *Tiphia popilliavora* Rohwer were introduced as biocontrol agents against Japanese beetles (*Popillia japonica* Newman) and oriental beetles (*Anomala orientalis* Waterhouse). Studies have shown that under field conditions, *T. vernalis* parasitize Japanese beetles more often than oriental beetles. This study was done to understand how tiphiid wasps handle the two different host species and the influence of host defensive behaviors on the oviposition process of tiphiid wasps. The preovipositional behaviors performed by *Tiphia* wasps included: stinging, examining, moving soil, kneading, host-feeding, and host scraping. The frequency, sequence, and total time spent on each behavior before oviposition were scored and compared between two host species. The sequence and frequency of preovipositional behaviors performed by both *Tiphia* wasps did not show a difference between the two host species. However, female *T. vernalis* spent significantly longer time trying to sting oriental beetles than Japanese beetles in order to paralyze them. The time *T. popilliavora* spent on prestinging behaviors did not show a difference between Japanese and oriental beetles. The defensive behaviors performed by Japanese and oriental beetle grubs included: vigorous movements, rubbing their abdomen or head against the wasp's abdomen, and biting at the attacking wasp. The frequency and total time spent on each defensive behavior was scored and compared between two host species. Overwintered, third instar oriental beetle grubs spent significantly longer time on defensive behaviors when they were attacked by *T. vernalis* which likely cause wasps to spend longer time trying to sting oriental beetle grubs. The active host resistance gained through behavioral defenses could make oriental beetle grubs less susceptible to *T. vernalis* attack than Japanese beetle grubs, especially under field conditions. Younger grubs attacked by *T. popilliavora* did not exhibit these differences.

1. Introduction

Two important scarab beetle species, the Japanese beetle (*Popillia japonica* Newman) and oriental beetle (*Anomala orientalis* Waterhouse) are considered invasive species and have been

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reported as key pests of urban landscapes and of various other agricultural settings in the Northeastern United States (US) (Köppenhofer and Fuzy, 2007; Vittum et al., 1999). The larvae of Japanese beetles primarily feed on the roots of a wide variety of plants, including all cool season grasses and most weeds that are commonly found in turfgrass sites. The root-feeding larvae of oriental beetles are a major pest of blueberries, ornamental nurseries, and turfgrass. During the late 1920s and early 1930s, the US Department of Agriculture (USDA) introduced several parasitoid wasp species in order to control the outbreak of Japanese beetles (Ramoutar and Legrand, 2007). Of these, two species of tiphiid wasps, *Tiphia vernalis* Rohwer and *Tiphia popilliavora* Rohwer were successfully established as biocontrol agents against Japanese beetle grubs (Ramoutar and Legrand, 2007). *T. vernalis* and *T. popilliavora* are also parasitoids of oriental beetles. *T. vernalis* attack overwintered third-instar larvae of Japanese and oriental beetles (Legrand, 2009; Rogers and Potter, 2002), *T. popilliavora* attack young third-instar and late second instar larvae of Japanese and oriental beetles (Clausen et al., 1927). Adult *T. vernalis* are active from the first week of May to the beginning of June with a peak around the last week of May, whereas adults of *T. popilliavora* emerge in August and early September (Legrand, 2009; Ramoutar and Legrand, 2007). Female *Tiphia* wasps, burrow into the soil and locate soil dwelling larval hosts using species-specific kairomones present in grub body odor trails and frass (Obeysekara and Legrand, 2011; Rogers and Potter, 2002). When a host is found, the wasp paralyzes it momentarily and attaches an egg in a location that is specific for the wasp species (Clausen et al., 1927).

Recent surveys in the US indicated a wide distribution of *T. vernalis* (Ramoutar and Legrand, 2007) and presence of *T. popilliavora* (Legrand, 2009) in Connecticut and other Northeastern states such as Massachusetts and New Hampshire (Legrand, 2012). Although, many *Tiphia* species are host specific a few studies have shown that some species may attack several co-occurring species of white grubs (Jaynes and Gardner, 1924; Legrand, 2009; Reding and Klein, 2007). The field parasitism rate on third-instar larvae of *P. japonica* and *A. orientalis* has been examined only for *T. vernalis*, parasitizing on average 53% *P. japonica* and up to 33% of *A. orientalis* in some selected towns of Connecticut (Legrand, 2009). In addition, recent field studies in Ohio nurseries by Reding and Klein (2007) found that 31% and 60% of the sampled *A. orientalis* and *P. japonica* populations, respectively, were parasitized by *T. vernalis*. Little is known about how tiphiid wasps handle different co-occuring scarab hosts and about factors involved in differential parasitism rates. The present work was motivated by the notion that the *Tiphia* parasitism rate difference between co-occuring scarab hosts could result from handling time differences before oviposition by the wasp and host defenses that can influence the host acceptance of parasitoids.

The behavioral events leading to oviposition by parasitic wasps play an important role in the effectiveness of insect parasitoids as biological control agents. Previous studies dealing with oviposition process of tiphiid wasps include a comprehensive account on preovipositional behaviors of *T. vernalis* and *Tiphia pygidialis* on white grubs by Rogers and Potter (2004) and a report on the oviposition behavior of *T. popilliavora* on *P. japonica* by (Clausen et al., 1927). Once a host is located tiphiid wasps were observed to perform a sequence of behaviors such as host examination, moving soil around the host, host kneading, host feeding and host scraping (Clausen et al., 1927; Rogers and Potter, 2004). No recent studies have examined the preovipositional behaviors of *T. popilliavora*. It is possible that *Tiphia* females would perform these preovipositional behaviors in a different sequence, frequency and duration when they attack the two host species, *A. orientalis* and *P. japonica*.

The host acceptance of tiphiid wasps has not been addressed in the literature. Host acceptance is the process whereby the host is detected and utilized by the parasitoid and can be influenced by several host-related factors including shape, size, movement, sound, and chemical cues (Harvey and Thompson, 1995; Vinson, 1976). Both third-instar *P. japonica* and *A. orientalis* grubs are almost equal in shape and size (Vittum et al., 1999). This suggests that the host acceptance might not be attributed to the size or the shape of grubs. Moreover, when offered a choice between grub body odor trails and frass from *P. japonica* and *A. orientalis*, female *Tiphia* wasps' responses to cues were not significantly different between the two host species (Obeysekara and Legrand, unpubl.). Several studies have shown that after contact, many hosts defend themselves by biting, writhing, trashing, regurgitating fluids aimed at the parasitoid, crawling, burrowing and by head or abdominal flicking and rearing (Godfray, 1993; Gross, 1993; Harvey and Thompson, 1995). *P. japonica* grubs were observed to perform aggressive and evasive behaviors such as brushing with the legs and rubbing with abrasive raster in responses to attack by entomopathogenic nematodes (Gaugler et al., 1994). Also, Rogers and Potter (2004) reported that *P. japonica* grubs show defensive behaviors in response to attacking *T. vernalis* such as rubbing their abdomen against the wasp's abdomen, biting at the attacking wasp, and burrowing away with initial contact with the wasps' antennae. Furthermore, observations had indicated that *A. orientalis* tend to be more defensive than *P. japonica* (Legrand, Pers. Obs.). Taken together, these findings indicate that the defensive behaviors of *P. japonica* and *A. orientalis* grubs in response to parasitoid attack may vary and could affect parasitism success of *Tiphia* wasps. Therefore, identification of host defensive strategies that could influence parasitism success may aid in explaining some of the variability in field parasitism rates (Georgis and Gaugler, 1991).

The effective use of tiphiid wasps in biological control programs for scarab pests is contingent on understanding how the wasps and their target hosts interact within the grass habitat. There are continuing efforts on finding new species of *Tiphia* in their native range and also on redistributing *T. vernalis* to areas of U.S. (e.g., Michigan, Wisconsin) into which *P. japonica* recently has spread (Rogers, 2003; Smitley, 2002). Future efforts using *Tiphia* wasps for *P. japonica* and *A. orientalis* management require understanding on the interactions between tiphiid wasps and their target hosts. To our knowledge, no empirical study has examined the behavioral defenses of scarabaeid grubs against attacking tiphiid wasps and the possible effects of those behaviors on parasitism success. Therefore the aims of this study were: (1) to examine the oviposition process of *T. popilliavora* on *P. japonica* and *A. orientalis* based upon videotaped oviposition events, (2) to determine whether the sequence, duration, and frequency of preovipositional behaviors of *T. vernalis* and *T. popilliavora* vary when the wasps attack *P. japonica* versus *A. orientalis*, and (3) to determine whether the duration and frequency of defensive behaviors of *P. japonica* and *A. orientalis* vary when they are attacked by *T. vernalis* and *T. popilliavora*.

2. Materials and methods

2.1. Collection and handling of wasps and grubs

T. vernalis and *T. popilliavora* were collected from turf areas at University of Connecticut Depot campus. *T. vernalis* were collected during late May 2012, by spraying 10% sugar water solution to the foliage of trees bordering areas of turfgrass. *T. popilliavora* were collected during August to early September 2012, from wild carrot (*Daucus carota*) flowers. The wasps were separated by gender, and females were placed individually into 118 ml plastic vials half filled with moist autoclaved soil. A piece of cotton wool soaked in a 10% sugar water solution was placed on the surface of the soil as a