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Authors: Boyd, David W., and Held, David W.

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Development of *Thripastichus gentilei* (Hymenoptera: Eulophidae) in the thrips *Gynaikothrips uzeli* (Thysanoptera: Phlaeothripidae)

David W. Boyd, Jr.[1] *and David W. Held*[2,*]

Abstract

Gynaikothrips uzeli (Zimmermann) (Thysanoptera: Phlaeothripidae) adults induce permanent leaf folds on *Ficus benjamina* L. (Moraceae), inside which they feed and reproduce. Range expansion of *G. uzeli* in North America was accompanied by *Thripastichus gentilei* (del Guercio) (Hymenoptera: Eulophidae). The objectives of this study were to determine the life stage(s) of *G. uzeli* parasitized by *T. gentilei*, the emergence period of *T. gentilei* from parasitized thrips, and the adult longevity of *T. gentilei*. We also determined the development time for both parasitoid and host at a constant temperature. Leaf folds representing only 1 life stage of *G. uzeli* were challenged with *T. gentilei* adults to determine life stage susceptibility. In a growth chamber at 30 °C, we recorded the development time for the thrips host and observed the emergence period of *T. gentilei* from thrips. Wasps were provisioned with liquid diets to determine longevity. Development of *G. uzeli* required 10.3 d at 30 °C with successful parasitism of only 1st and 2nd instars of the thrips. Wasps emerged from thrips about 20 d after their exposure to females and could survive in the laboratory for about 3 d longer when provisioned with sugar solution relative to water only or starved wasps. We concluded that *T. gentilei*, released for control of *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae) is now also using *G. uzeli* as a host. This larval parasitoid takes about twice as long as *G. uzeli* to develop, yet adults can survive up to 16 d when a carbohydrate source is present. *Thripastichus gentilei* should complement the activity of other natural enemies attacking *Gynaikothrips* species on *Ficus.* However, the development rate of *G. uzeli* and its potential interactions with *Montandoniola confusa* Streito & Matocq (Hemiptera: Anthocoridae) may limit the ability of *T. gentilei* to establish on plants wherever this anthocorid is present.

Key Words: biological control; development; weeping fig

Resumen

Los adultos de *Gynaikothrips uzeli* (Zimmermann) (Thysanoptera: Phlaeothripidae) inducen el doblamiento permanente en las hojas de *Ficus benjamina* L. (Moraceae) en cuyo interior se alimentan y reproducen. La expansión del rango geográfico de *G. uzeli* en América del Norte fue acompañado por *Thripastichus gentilei* (del Guercio) (Hymenoptera: Eulophidae). Los objetivos de este estudio fueron determinar el estadio(s) de vida de *G. uzeli* parasitado por *T. gentilei*, el período de emergencia de *T. gentilei* de los trips parasitados, y la longevidad de los adultos de *T. gentilei*. También se determinó el tiempo de desarrollo del parasitoide y del hospedero a una temperatura constante. Las hojas dobladas que representa sólo un estadio de vida de *G. uzeli* fueron expuestas a los adultos de *T. gentilei* para determinar la susceptibilidad de los estadios de vida. En una cámara de crecimiento a 30 °C, se registró el tiempo de desarrollo del hospedero de los trips y observamos el período de emergencia de *T. gentilei* de los trips. Se aprovisionan las avispas con dietas líquidas para determinar la longevidad. El desarrollo de *G. uzeli* requiere 10.3 días a 30°C con un parasitismo exitoso en sólo el primer y segundo instar de los trips. Las avispas salieron de los trips unos 20 días después de estar expuestas a las hembras y pueden sobrevivir en el laboratorio por unos 3 días más cuando aprovisionado con una solución de azúcar en relación con solamente agua o avispas hambrientas. Llegamos a la conclusión de que *T. gentilei*, liberado para el control de *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae) ahora también esta utilizando a *G. uzeli* como hospedero. Este parasitoide larval tarda alrededor de dos veces más tiempo que *G. uzeli* para desarrollarse aún que los adultos pueden sobrevivir hasta 16 días en una fuente de hidratos de carbono cuando está presente. El *Thripastichus gentilei* debe complementar la actividad con otros enemigos naturales que atacan *Gynaikothrips* spp. sobre *Ficus*. Sin embargo, la tasa de desarrollo de *G*. *uzeli* y sus posibles interacciones con *Montandoniola confusa* Streito & Matocq (Hemiptera: Anthocoridae) puede limitar la capacidad de *T. gentilei* para establecer en las plantas donde este antocorido está presente.

Palabras Clave: control biológico; desarrollo; higo llorón

Weeping fig, *Ficus benjamina* L. (Moraceae), is a popular plant used in interiorscapes, conservatories, or in outdoor landscapes in tropical areas in the United States. Adult *Gynaikothrips uzeli* (Zimmermann) (Thysanoptera: Phlaeothripidae) feed on young, developing leaves of weeping fig inducing the leaf to fold along the mid-vein forming a gall (Vardarasan & Ananthakrishnan 1981; Held et al. 2005; Arthurs et al. 2011). Galls induced by *Gynaikothrips* form in less than 24 h, and folded leaves do not recover (Paine 1992) and often abscise (Arthurs et al. 2011). *Gynaikothrips uzeli* feeds and reproduces inside foliar galls induced on weeping fig. Originally from southeast Asia, *G. uzeli* was first confirmed in the United States in 2003 and is now reported from landscapes, greenhouses, and interiorscapes in Alabama, Connecticut,

[1]Bob Jones University, Department of Biology, Greenville, South Carolina 29614, USA; E-mail: dboyd@bju.edu (D. W. B. Jr)
[2]Auburn University, Department of Entomology and Plant Pathology, Auburn, Alabama 36849, USA; E-mail: dwh0004@auburn.edu (D. W. H.)
*Corresponding author; E-mail: dwh0004@auburn.edu (D. W. H.)

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Florida, Indiana, Louisiana, Mississippi, Missouri, Tennessee, Texas, and Wisconsin (Held et al. 2005; Held & Boyd 2008b; Boyd & Held, unpublished). Much of the movement in the southeastern United States has been attributed to the horticulture trade (Held et al. 2005).

At least 3 natural enemies specific to gall-inhabiting thrips are established in the United States: *Androthrips ramachandrai* Karny (Thysanoptera: Phlaeothripidae) (Boyd & Held 2006), *Montandoniola confusa* (= *moraguesi*) Streito & Matocq (Hemiptera: Anthocoridae) (Dobbs & Boyd 2006), and *Thripastichus gentilei* (del Guercio) (Hymenoptera: Eulophidae) (LaSalle 1993). Following introduction of Cuban laurel thrips (*Gynaikothrips ficorum* [Marchal]; Thysanoptera: Phlaeothripidae), *M. confusa* and *T. gentilei* were released in the United States (LaSalle 1993; Dobbs & Boyd 2006). Both *M. moraguesi* and *T. gentilei* have been distributed throughout the southeastern United States coincident with weeping fig infested with *G. uzeli* (Held et al. 2005; Dobbs & Boyd 2006; Arthurs et al. 2011).

Thripastichus gentilei, the only species in the genus *Thripastichus* (monotypic), is a parasitoid of thrips recorded from species of *Gynaikothrips*, *Hoplothrips*, *Schedothrips*, *Mallothrips*, and *Liothrips* (Bournier 1967; Ananthakrishnan & Swaminathan 1977; Varadarasan & Ananthakrishnan 1981; LaSalle 1993; Loomans & van Lenteren 1995). It also attacks other gall-inhabiting, predatory thrips such as *A. ramachandrai* (Varadarasan & Ananthakrishnan 1981; Boyd & Held 2006). In North America, all adults of *T. gentilei* are females (Burks 1943) and can be monitored with sticky traps. Wasps and thrips are diurnal with peak activity in the afternoon (Held & Boyd 2008a).

Females of *T. gentilei* are 1.0 to 1.4 mm long, and larvae develop as endoparasitoids. Across host thrips species, the life stages that are reportedly parasitized by *T. gentilei* vary significantly (Burks 1943; Bennett 1965; Bournier 1967; Ananthakrishnan & Swaminathan 1977; LaSalle 1993; Loomans & van Lenteren 1995). These reports, however, list no records of the host life stage of *G. uzeli* used by *T. gentilei* or developmental data for either thrips or wasp species. Eggs of *T. gentilei* in *Liothrips oleae* Costa (Thysanoptera: Phlaeothripidae) will hatch in 2 to 3 d, develop for 8 to 10 d, and then emerge from thrips mummies (swollen, parasitized immatures; Fig. 1) (Bennett 1965; Bournier 1967).

Our objectives were to determine the life stage(s) of *G. uzeli* parasitized by *T. gentilei*, the development period at a constant temperature, and the longevity of *T. gentilei* adults. These objectives were identified to provide data to support releases of *T. gentilei* at the Audubon Aquarium of the Americas Amazon display, New Orleans, Louisiana. For this reason, we also measured the productivity of rearing *T. gentilei* adults from parasitized thrips as an alternative to just releasing adults for biological control. This interiorscape has many large *F. benjamina* trees infested with *G. uzeli*, and natural enemies were the only allowable option for control.



Fig. 1. Two immatures of *Gynaikothrips uzeli*: the upper individual is a normal larva, and the lower one displays the transparent, swollen appearance of a parasitized larva. The parasitoid, *Thripistichus gentilei*, develops with a polarity that is reverse relative to the host.

Materials and Methods

SOURCES OF INSECTS

Ficus benjamina plants infested with *G. uzeli* were obtained from local retail nurseries in Mississippi (Harrison and Pearl River Counties). Initial populations of *T. gentilei* were obtained from Alabama (Mobile Co.) on a weeping fig tree heavily infested with *G. uzeli*. Populations of both insects were maintained in greenhouses at the United States Department of Agriculture (USDA) Southern Horticultural Laboratory in Poplarville, Mississippi. All experiments used only newly emerged (<24 h old) wasps reared from pupae inside leaf galls. Voucher specimens of *G. uzeli* and *T. gentilei* were deposited in the National Museum of Natural History, Washington, District of Columbia.

LIFE STAGE SUSCEPTIBILITY OF *G. UZELI* TO *T. GENTILEI*

Experiments were conducted at the USDA Southern Horticultural Laboratory. Seventy-two cuttings of *F. benjamina* with at least 1 developing terminal leaf for oviposition were harvested from pesticide-free plants and taken into the laboratory. Cuttings were prepared individually in small portion cups by using the technique described by Held & Boyd (2008a). Ten adults of *G. uzeli* were added to each cutting and placed in an incubator (I-30BLL, Percival Scientific, Boone, Iowa) at 30 ± 0.5 °C, 60 ± 10% RH, and a 16:8 h L:D photoperiod for 5 d. Development time of both parasitoid and host were determined at 30 °C, which is the temperature maintained in the exhibit where *T. gentilei* was to be released.

After adult thrips were removed, the number of eggs present was counted and the progression of development was checked daily by using morphological features described by Lewis (1973). The presence of wing buds distinguished the prepupal and pupal stages from 1st and 2nd instars, which can be separated by size. Pupa II stages have a darker coloration, which distinguished them from individuals in the pupa I stage. Fifty cuttings with ≥50 eggs were used for the *T. gentilei* experiments. A 2nd set of 22 cuttings, with 10 to 48 eggs each, were used to monitor development of *G. uzeli.* The development time for each life stage and time from hatch to adult emergence was averaged across all replicates. The exact date of oviposition could not be determined because galls must be induced before oviposition (Varadarasan & Ananthakrishnan 1981). With an aggregation of thrips, gall induction occurs within 1 d followed by oviposition within a few days after gall induction (Boyd & Held, personal observations). Cuttings were excluded from the data set if the gall abscised. Percentage of egg hatch was calculated for eggs in 17 galls but only replicates (n = 9) where ≥50% of immatures developed to adults were included in the calculations of development time.

For experiments with *T. gentilei*, cuttings were sorted into replicates so that cuttings within a replicate had a similar number of eggs. Within a replicate, cuttings were randomly assigned a life stage treatment (1st instar, 2nd instar, prepupa, pupa I, and pupa II) similar to that of Tavares et al. (2013). To achieve uniform life stages, immatures were allowed to develop from eggs inside leaf galls to a certain stage; then all individuals not at that prescribed stage were destroyed. However,

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preliminary data forced the grouping of the prepupa, pupa I, and pupa II stages into a single generic "pupa" category because these stages, particularly the prepupa stage, were too short (≤24 h). A single *T. gentilei* wasp was added to each cutting and provided with a 0.1 M sucrose solution on a cotton ball. After 2 d, wasps were removed from the cuttings and replicates were excluded if the wasp was dead. The number of parasitoid mummies and the number of emerged adults were counted at 7, 10, and 20 d. The introduction of the wasp was counted as the initial day because it was not possible to observe oviposition.

Because a long-term project goal was to release *T. gentilei* for biological control, we wanted to know if the introduction of parasitized thrips would minimize the need for rearing and release of adults. Therefore, an experiment was conducted to estimate the number of wasps produced relative to weight of parasitized thrips and to determine the emergence period from parasitized thrips. A 0.3 g sample size of parasitized thrips was collected from leaf galls. Parasitized thrips can be easily sorted from exuvia by the appearance of the wasp pupa inside the nearly translucent integument of the thrips host (Fig. 1). These wasp pupae were of mixed age at the time of collection. Parasitized thrips were weighed and placed into 8 dram vials and sealed with a moistened cotton ball. Sixteen vials were prepared for each weight and held in a growth chamber at 30 °C for 28 d. Vials were checked for wasp emergence every 2 d for 28 d, and the cumulative number of wasps was recorded and standardized per 100 mg of parasitized thrips.

EFFECT OF DIET ON WASP LONGEVITY

This experiment, conducted in the laboratory at the Mississippi State University Coastal Research and Extension Center, Biloxi, Mississippi, determined the longevity of *T. gentilei* provisioned with various diets and thrips hosts. A cotton ball with 1 of 3 treatments—0.1 M sucrose, distilled water, or no food (starved)—was placed in a 355 mL, translucent plastic cup along with a galled cutting of *F. benjamina* containing immature *G. uzeli*. One newly emerged *T. gentilei* adult was released in the cup, and another 355 mL, translucent plastic cup with a mesh-covered hole in the bottom was placed on top and sealed with parafilm. Cups with wasps were maintained in the laboratory under fluorescent lighting with a 16:8 h L:D photoperiod at 23 °C.

Wasps were checked every 2 d and, if still alive, were moved to another cup with the respective treatment and an infested cutting. To minimize handling mortality, wasps were allowed to either walk or fly into the new cup. Each treatment was replicated 3 times in each of 4 separate trials (12 total replicates). Data were pooled across all trials and survival summarized as Kaplan–Meier survivorship functions and analyzed using the Peto–Wilcoxon test (Statistix, Analytical Software, Tallahassee, Florida). Kaplan–Meier survivorship function percentiles were used to calculate median survival time (d) for each treatment.

Results

LIFE STAGE SUSCEPTIBILITY OF *G. UZELI* TO *T. GENTILEI*

At 30 °C, *G. uzeli* egg hatch averaged 73.5 ± 4.4% and development from 1st instar to adult lasted 10.3 ± 0.2 d (Table 1). Both 1st and 2nd instar stages required >2 d at 30 °C with subsequent stages <2 d each. Wasps probe all immature stages and even dead immatures with the ovipositor (D. Boyd, personal observations). Successful development of *T. gentilei* was evident and recorded once mummies (wasp pupae inside thrips hosts) were visible. Although pupa I and II stages (n = 6) were exposed to *T. gentilei* for oviposition, mummies or adult wasps did not develop in those life stages up to 23 d after exposure to the wasp.

Table 1. Development periods of *Gynaikothrips uzeli* life stages at 30 °C, 60% RH, and a 16:8 h L:D photoperiod.

Stage	Average (± SE) development time (d)
1st instar	2.9 ± 0.2
2nd instar	3.2 ± 0.3
Prepupa	0.8 ± 0.1
Pupa I	1.4 ± 0.2
Pupa II	1.9 ± 0.2

Successful development was not evident until 10 d after immature *G. uzeli* were exposed to *T. gentilei.* At day 10, 99 thrips mummies were recorded for 1st instar treatments (46% of individuals exposed) and 51 thrips mummies (11% of individuals exposed) developed when 2nd instars of *G. uzeli* were exposed. At 30 °C, wasps completed development and pupated after 8 to 10 d in *G. uzeli*, and they began to emerge from parasitized thrips 19 d after larvae had been exposed to wasps for oviposition. Based on our collections of mixed-age wasp pupae inside parasitized *G. uzeli* hosts, an average of 6 wasps (range 3–8) were produced per 100 mg sample of thrips mummies.

EFFECT OF FOOD PROVISION ON WASP LONGEVITY

A maximum lifespan of 16 d was observed for adults of *T. gentilei.* Median survival time of 11 d for wasps provided sugar water was significantly greater than for those given either water or starved (χ^2 = 15.45, df = 2, P < 0.001; Fig. 2). Survival times of wasps provisioned with water or starved were not different.

Discussion

These experiments were designed to support our efforts to manage *G. uzeli* in an interiorscape by using *T. gentilei.* We were interested in wasp longevity, susceptibility of different life stages, and how wasp development compared with development of the thrips host. In the presence of thrips hosts, supplemental sugar solutions significantly increased adult longevity by about 3 d. We provided thrips hosts in all assays so the possibility existed for host feeding, although this behavior is not mentioned in any previous work (Ananthakrishnan & Swaminathan 1977; Loomans & van Lenteren

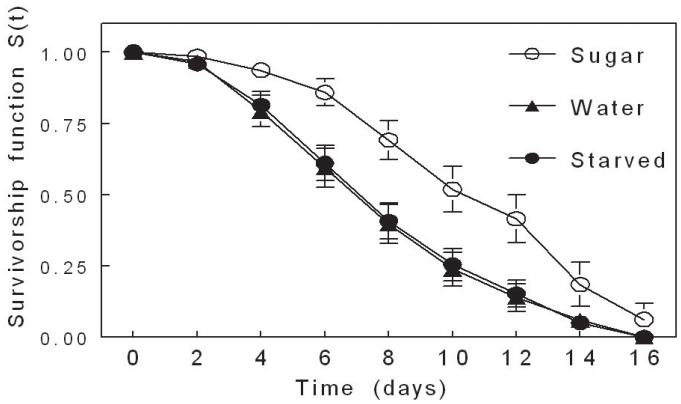


Fig. 2. Survivorship function [S(t)] for *Thripistichus gentilei* adults provisioned with a sugar water solution, distilled water, or no food (starved) along with thrips hosts. Median survival time was greater for wasps provided sugar water compared with survival for those given either water or starved. Bars represent the standard error [SE S(t)].

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1995). The 11 d median life span for adults of *T. gentilei* reported here is considerably longer than the 48 to 72 h laboratory longevity when wasps were provisioned with 2% honey (Ananthakrishnan & Swaminathan 1977). Caged adults in field conditions, however, can live 10 d (Loomans & van Lenteren 1995). Wasps able to find supplemental sugar, perhaps as honeydew or nectar, will live longer and potentially show enhanced parasitism of thrips. Weeping fig is a common host for many families of Sternorrhyncha, and soft scales and mealybugs were present on trees in the interiorscape where *T. gentilei* was released. Mealybugs are also a common inquiline inside galls produced by *Gynaikothrips* and may be a source of honeydew for *T. gentilei* searching inside galls (Tawfik 1967; Held et al. 2005).

Wasps were observed to probe all immature stages, but completion of development (production of adults) was limited to 1st and 2nd instar hosts in our experiment. These results do not exclude the possibility that oviposition occurred in other life stages. Developmentally, the 1st and 2nd instars represent about 60% of the post-egg hatch development time, but immatures are not always the dominant life stage inside galls (Paine 1992; Held et al. 2008). Wasps likely have to move between galls to locate suitable hosts. Wasps disperse from galls by walking or flying, and detection on sticky cards (Held & Boyd 2008a) suggests that searching for suitable life stages may occur between plants. Based on observations and controlled experiments, wasps emerge from thrips hosts after 19 d, approximately twice as long as the development of *G. uzeli.* At 30 °C, *G. uzeli* development was faster than the 16 d development time for related Cuban laurel thrips at the same temperature (Paine 1992). We did not evaluate different temperatures for development of *G. uzeli*; however, development would be predictably slower at cooler temperatures. For example, Cuban laurel thrips require 48 d to complete development at 15 °C (Paine 1992). The slow growth–high mortality hypothesis (Clancy & Price 1987) predicts that slower development of the host thrips would favor success by *T. gentilei*. Therefore, populations of *T. gentilei* may be more abundant in cooler parts of the year (i.e., spring and fall). This assumption is consistent with the observation by Bournier (1967) that parasitism of *G. ficorum* by *T. gentilei* is greatest (up to 75%) in the fall.

In the interiorscape in which we were working, *T. gentilei* was released as parasitized pupa and adult wasps by using an inoculative method. Populations of thrips and wasps could then be checked regularly using sticky cards (Held & Boyd 2008a) or by direct inspection of galls for thrips mummies. At the time of this study, the anthocorid predator *M. confusa* was also available in our laboratory colony, but this insect was not used for inoculative releases. In North America, many populations of *T. gentilei* overlap with *M. confusa* (Dobbs & Boyd 2006; Arthurs et al. 2011). Adults of *M. confusa* prefer eggs of both *G. uzeli* and *G. ficorum* as prey, yet larval consumption is common and may increase when eggs are not available (Arthurs et al. 2011; Tavares et al. 2013). Based on the preferred life stage for each of these 2 natural enemies, they would seem to act complementarily. However, the anthocorid will attack *T. gentilei* adults (Fig. 3) and may indiscriminately consume parasitized thrips. Previous work would suggest that *M. confusa* is a more effective natural enemy at initially high thrips densities reducing leaf galls and thrips populations in about 5 wk (Arthurs et al. 2011). Alternatively, low populations of thrips (such as isolated infested plants in an interiorscape) may be a better situation for inoculative release of *T. gentilei*. There remains a potential for the anthocorid to hinder establishment of *T. gentilei*, and this interaction should be considered when both natural enemies are present or are being considered for releases in biological control programs.



Fig. 3. *Montandoniola confusa* (Anthocoridae) attacking *Thripistichus gentilei*. This predator also feeds on the eggs and immature stages of *Gynaikothrips* species.

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