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Source: Florida Entomologist, 100(1) : 52-56

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.100.0109>

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# Seasonal abundance of the adventive Chinese tallowtree herbivore *Caloptilia triadicae* (Lepidoptera: Gracillariidae) and its parasitoids

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## Abstract

Chinese tallowtree, *Triadica sebifera* (L.) Small (Malpighiales: Euphorbiaceae), is an invasive weed from southern China that is invasive in the Gulf states of the southeastern USA. One significant factor that contributes to the success of this weed has been the lack of herbivore species attacking it in the invaded range. However, the leafminer species *Caloptilia triadicae* Davis (Lepidoptera: Gracillariidae) was discovered feeding on Chinese tallowtree in Florida in 2008 and has now been found throughout much of the plant's invaded range. We monitored the seasonal abundance of *C. triadicae* populations and their associated parasitoids over 2 yr in Florida. Populations of *C. triadicae* peaked in Jun and Jul in 2013 and 2014. Parasitism averaged about 3.3% of larvae and pupae, with a peak of 12%. The most abundant parasitoid species was *Goniozus* sp. (Hymenoptera: Bethyridae) (76% of all parasitoid individuals reared) and, second, *Brasema* sp. (Hymenoptera: Eupelmidae) (18% of parasitoids). Due to specific niche requirements and the apparent exploitation of leaf mines by these parasitoid species, it is doubtful they will attack species being considered for biological control of Chinese tallowtree.

Key Words: biological control of weeds; *Triadica sebifera*; adventive species; invasive weed

## Resumen

El árbol de sebo chino, *Triadica sebifera* (L.) Small (Malpighiales: Euphorbiaceae), es una maleza invasora del sur de China que es invasiva en los estados del Golfo del sureste de Estados Unidos. Un factor significativo que contribuye al éxito de esta mala hierba ha sido la falta de especies herbívoras que la atacan en el área de invasión. Sin embargo, una especie de minador de hojas *Caloptilia triadicae* Davis (Lepidoptera: Gracillariidae) fue descubierta alimentándose del árbol de sebo chino en la Florida en el 2008 y ahora se la ha encontrado a través de gran parte del rango invadido de la planta. Se monitoreó la abundancia estacional de poblaciones de *C. triadicae* y sus asociados parasitoides por un período de 2 años en la Florida. Las poblaciones de *C. triadicae* alcanzaron su máximo en junio y julio del 2013 y del 2014. El promedio de parasitismo de larvas y pupas fue del 3,3%, con un pico de 12%. La especie de parasitoide más abundante fue *Goniozus* sp. (Hymenoptera: Bethyridae) (76% de todos los individuos parasitoides criados) y, segundo *Brasema* sp. (Hymenoptera: Eupelmidae) (18% de los parasitoides). Debido a los requerimientos de nichos específicos y a la aparente explotación de las minas de hojas por estas especies de parasitoides, es dudoso que ellos atacarán a las especies que se consideran para el control biológico del árbol de sebo chino.

Palabras Clave: control biológico de malezas; *Triadica sebifera*; Gracillariidae; especies adventivas; maleza invasora

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Plant invasions have broad impacts on the composition of invaded communities (Mooney & Cleland 2001). Invasive plants are a new resource that may be exploited by local species with diverse trophic relations to the plant (White et al. 2006). Some of these interactions are direct, such as those between a plant and associated herbivores, but others might be indirect, as with invasive plant species linked to a variety of higher trophic-level species assemblages (Veldtman et al. 2011). Indigenous natural enemies may take advantage of recent introductions of exotic insects, although at reduced levels compared with their attack of native hosts (Cornell & Hawkins 1993). These new arrivals may be introduced either accidentally or intentionally as biological control agents. Natural enemy antagonists have been linked to the prevention of establishment or reduction of the effectiveness of species introduced for biological control of weeds (Goeden & Louda

1976; Hill & Hulley 1995; Paynter et al. 2010). Native parasitoids often exploit introduced biological control agents soon after the agents' release (Cornell & Hawkins 1993; Hill & Hulley 1995; Paynter et al. 2010; Boughton et al. 2012). Knowledge of the patterns of parasitoid attack before and following agent release may assist in directing selection of future agents that are less prone to attack (Paynter et al. 2010).

Chinese tallowtree, *Triadica sebifera* (L.) Small (Malpighiales: Euphorbiaceae) (= *Sapium sebiferum*) (hereafter "tallow") is one of the most damaging invasive weeds in the southeastern USA, affecting wetlands, forests, and natural areas. Tallow is a deciduous tree that grows to 15 m tall in a wide range of forest types, on different soils, and under dry and moist conditions (Zheng et al. 2005). Currently, tallow infests 185,000 ha of southern forests, stranded swamps, flatwoods, and ru-

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deral communities in areas of 10 states that border the Gulf of Mexico and in California (EDDMapS 2015; Invasive.org 2015). Costs of likely future timber losses and control over the next 20 yr in forestlands of Texas, Louisiana, and Mississippi range from \$200 million to \$400 million (Wang HH et al. 2012).

Most of the herbivores that feed on tallow in the USA are generalists, including the grasshoppers *Melanoplus angustipennis* (Dodge) and *Orphulella pelidna* Burmeister (Orthoptera: Acrididae) (Siemann & Rogers 2003; Lankau et al. 2004). Experimental plantings of tallow in Florida are routinely damaged by generalists including the larvae of *Spodoptera frugiperda* Smith & Abbot and *Spodoptera latifascia* Walker (Lepidoptera: Noctuidae), as well as by adults of the invasive weevils *Mylocherus undatus* Marshall, *Pachnaeus litus* (Germar), and *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) (G. S. W. unpublished data). The only specialist herbivore feeding on tallow known from the USA is the moth *Caloptilia triadicae* Davis (Lepidoptera: Gracillariidae), whose larvae mine and form blotches on the leaves (Davis et al. 2013). This adventive species has been reported from nearly all tallow-infested areas of the southeastern USA (Fox et al. 2012). *Caloptilia triadicae* is most likely from Asia and is thought to have arrived in the USA between 2002 and 2004 (Fox et al. 2012). Populations of *C. triadicae* were found in Florida in 2008 feeding on tallow trees planted to support biological control efforts of this invasive weed (Davis et al. 2013). Since this initial discovery, this insect has been found commonly in tallow-infested areas of Florida from early summer to Nov. During the early instars, larvae are sap feeding and form serpentine mines in young leaves. Later instars emerge from the mine and feed externally constructing a coiled, rosette-shaped leaf whorl (Fox et al. 2012; Davis et al. 2013). Mines occur on new growth, and some tallow saplings appear heavily damaged. Although *C. triadicae* was not introduced intentionally as a biological control agent, it shows narrow specificity completing development on only 1 other species besides the target weed (Duncan et al. 2016). Our goals were to document the seasonal abundance of this leafminer over 2 growing seasons on tallow in Florida and to document the diversity and abundance of any parasitoids attacking this herbivore's larvae or pupae.

## Materials and Methods

### SITES

To examine seasonal dynamics of *C. triadicae*, all instars and their parasitoids were monitored in a patch of planted tallow (hereafter "garden") infested with *C. triadicae* near Ft. Lauderdale (Broward County; 26.084611°N, 80.240384°W), Florida, in 2013 and 2014. To supplement these collections, all instars and pupae of *C. triadicae* were collected in leaf whorls near Leesburg (Lake County; 28.93655°N, 81.79298°W), Gainesville (Alachua County; 29.58702°N, 82.36225°W), Tallahassee (Leon County; 30.475980°N, 84.182896°W, and 30.463672°N, 84.182167°W), and West Augustine (St John's County; 29.91619°N, 81.37440°W), Florida. All sites were infested with tallow trees that had been present for more than 20 yr.

### PLANTS

Tallow plants were grown at the United States Department of Agriculture/Agricultural Research Service (USDA/ARS) Invasive Plant Research laboratory in Ft. Lauderdale, Florida, from seeds collected in Gainesville, Florida, in 2010. Their waxy coats were removed by soaking in water with laundry detergent (10 g/L) for 2 d. The prepared seeds were stored frozen (−10 °C) for 8 wk and then planted 1 cm deep in

germinating media (Fafard Germinating Mix formula, Sun Gro Horticulture, Agawam, Massachusetts). Germination occurred in a screen-house, under a 14:10 h L:D photoperiod produced by use of overhead lights (Spectralux T5 HO 54 Watt lights, Sun Blaze T5HO-48 fixtures, Sunlight Supply Inc., Vancouver, Washington). Daytime and nighttime temperatures averaged 25° C and 17° C, respectively. When the plants ( $n = 50$ ) were approximately 30 to 45 cm tall, they were transferred to 3.7 or 11 L pots in potting media (Fafard 3B Professional formula Potting Mix, Sun Gro Horticulture, Agawam, MA) and moved to an outside garden. Drip irrigation was provided with 3.66 L per pot twice daily. Plants were fertilized every 2 wk with Peters Professional 20-20-20 (The Scotts Company LLC, Marysville, Ohio) at label rate and twice per year with controlled release fertilizer (Everris Nursery Mix, The Scotts Company LLC, Marysville, Ohio; 21-4-8, 22.5 g). No insecticides were applied to the plants. During insect collections, these plants were generally 1 to 1.5 m tall and had abundant newly flushed foliage.

### INSECTS

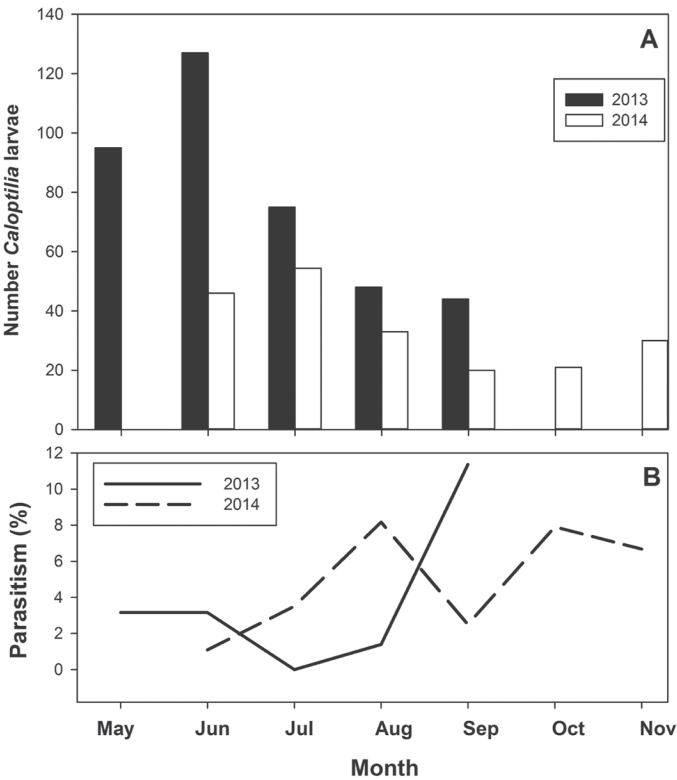
To document field populations and seasonal dynamics of *C. triadicae*, leaf whorls were collected monthly as available during 2013 and 2014 in the tallow garden described above at Ft. Lauderdale, Florida. During each sampling, *C. triadicae* whorls were collected for 1 h by 1 collector and brought to the laboratory. The presence of live larvae or pupae was confirmed in each whorl by observation with a stereomicroscope (7.1x magnification). Whorls were set up individually in small Petri dishes (5.5 cm diameter) lined with moistened filter paper and sealed to the base of the Petri dish with a cotton string. Whorls were placed on a laboratory bench top at a 13:11 h L:D photoperiod and 25 °C temperature. Data were collected on the numbers of whorls collected and the numbers of *C. triadicae* adults and parasitoids that emerged.

Parasitoid species diversity was assessed by collecting *C. triadicae* larvae-infested whorls in bulk (about 250 g) from Ft Lauderdale, Gainesville, Tallahassee, and West Augustine, Florida. Whorls were placed in a large Petri dish (20 × 7 cm) lined with moistened paper. The species of parasitoids that emerged were collected and stored in 95% alcohol for identification.

## Results

Host *C. triadicae* larvae were first sampled in May of 2013, and their density (as number per man-hour of collection effort) peaked during Jun and Jul of 2013 and 2014 (Fig. 1). Following these peaks, the density of *C. triadicae* larvae and pupae decreased until none was found in Oct 2013 or in Nov 2014. In total, 1,534 larvae and pupae were collected, 940 during 2013 and 594 in 2014 (Fig. 1). Adult leafminers emerged from 55.5% of these larvae or pupae. No larvae or pupae were found between Nov and May of each year as the deciduous tallow leaves were unavailable.

Parasitism occurred each year from Jun to Sep (Fig. 1). During the 2 yr of data collection, parasitism never exceeded 12% of collected larvae and pupae. Overall parasitism was 3.3%, and the most common parasitoids were a *Goniozus* sp. (Hymenoptera: Bethyliidae) (76% of parasitoids), a *Brasema* sp. (Hymenoptera: Eupelmidae) (18% of parasitoids), and a *Sympiesis* sp. (Hymenoptera: Eulophidae) (6% of parasitoids) (Table 1; Fig. 2). Two additional parasitoid species noted were *Zagrammosoma multilineatum* (Ashmead) (Hymenoptera: Eulophidae) and either *Euplectrus* or *Platyplectrus* sp. (Hymenoptera: Eulophidae). When a host larva was parasitized by *Goniozus* sp., 3 parasitoids typically emerged from the host, whereas all of the other species were solitary.



**Fig. 1.** *Caloptilia triadicae* larval and pupal population dynamics (A) and percentage of parasitism (B) from tallow grown in a garden in Ft. Lauderdale, Florida. Larvae and pupae were collected in leaf whorls.

Discussion

We found that several parasitoids attacked a relatively new immigrant herbivore that has only been present in North America for about 10 yr (Fox et al. 2012). A similar parasitism pattern was found for the introduced biological control agent *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae) on *Lygodium microphyllum* (Cav.) R. Br. (Schizaeales: Lygodiaceae) (Kula et al. 2010; Boughton et al. 2012). In south Florida, 6 parasitoid species were discovered exploiting *N. conspurcatalis* parasitizing 6.8% of the field-collected larvae. Apparently, such novel hosts may expect only a brief period of enemy-free space before they are discovered and exploited by native natural enemies (Carvalho et al. 2008; Paynter et al. 2010). Over time, the diversity and density of parasitoid species exploiting the tallow leafminer in Florida is likely to increase due to its further spread or behavioral adaptation by local parasitoids (Grabenweger et al. 2010).

The parasitoids we found attacking this tallow leafminer were most likely generalists that occupied habitats near the tallow-invaded area (Cornell & Hawkins 1993). These parasitoids probably exploited

hosts that feed as leafminers or species from other guilds with similar feeding habits, similar to that of the tallow-feeding *C. triadicae* larvae. Leafminers attacking cultivated citrus may be one source of these *C. triadicae* parasitoids in Florida as some of our collection sites were near the large citrus-growing areas that support the exotic citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Native generalists, primarily from the eulophids, attack the exotic citrus leafminer *P. citrella* in Florida (Peña et al. 1996; Amalin et al. 2002; Xiao et al. 2007). Our results show that at least 1 of the parasitoids associated with *P. citrella*, *Z. multilineatum*, was also recovered from the tallow-feeding leafminer *C. triadicae*. The introduced *P. citrella* parasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was not recovered in our surveys.

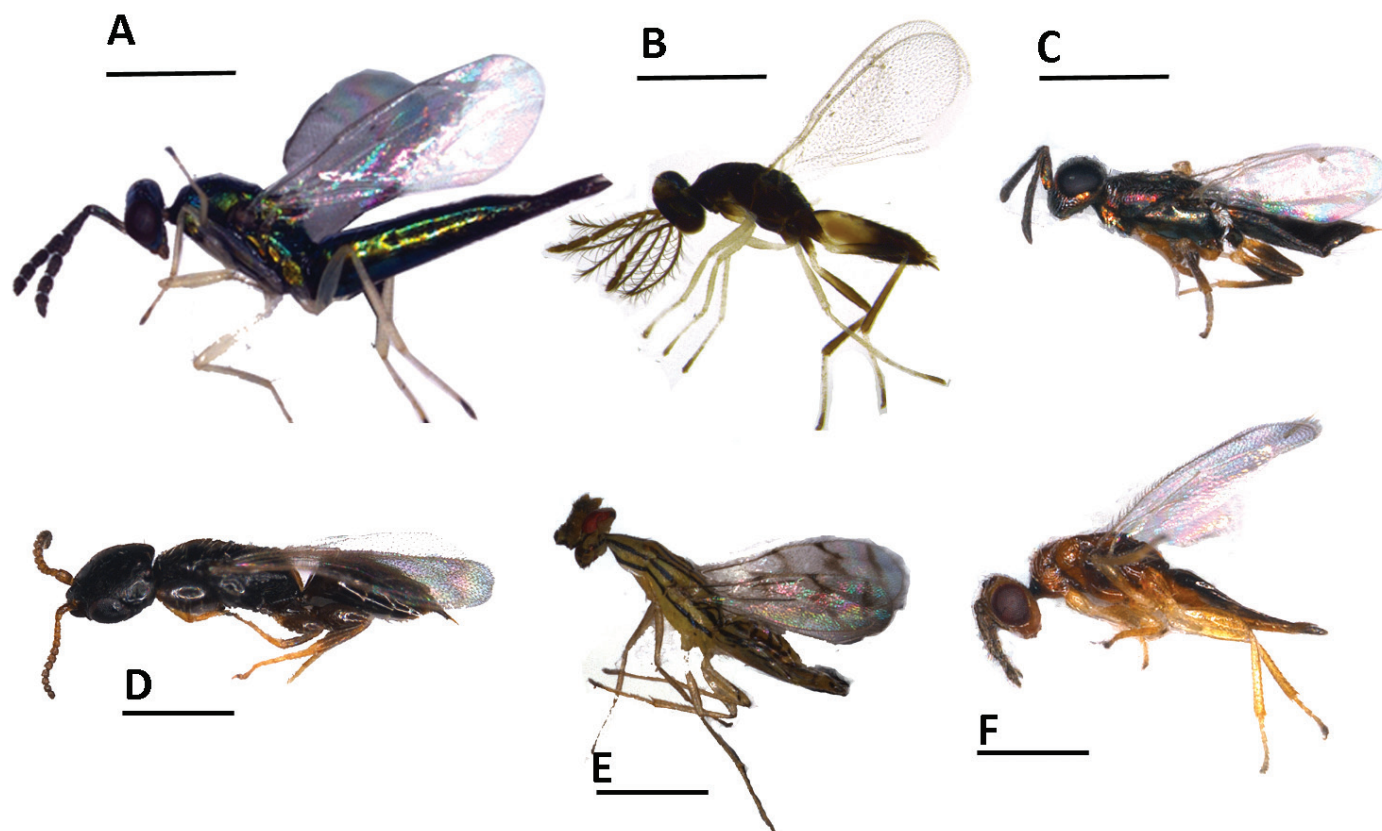
Although all these parasitoids are thought to be native and to probably attack many species, their origin is unclear. However, they all attack hosts from similar feeding guilds. *Brasema* species are mostly primary or secondary larval/pupal ectoparasitoids of a wide variety of holometabolous insects in concealed situations (Gibson 2011). *Goniozus* species are ectoparasitoids of immature stages of microlepidopteran families (e.g., Gelechiidae, Pyralidae, and Tortricidae) (Gordh & Móczár 1990). *Goniozus* species are known to parasitize several species that occupy concealed feeding locations, such as *Platynota idaeusalis* Walker (Lepidoptera: Tortricidae) (Brown et al. 2010), and European grapevine moth, *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae) (Moreau et al. 2010). Malaise trap surveys in central Florida detected 9 species of *Goniozus* (Evans & Fullerton 1997). Species of *Sympiesis* frequently exploit gracillariid leafminers (Grabenweger & Lethmayer 1999; Mafi & Ohbayashi 2006), including the citrus leafminer *P. citrella* (Schauff et al. 1998), or tortricid leafrollers (Cossentine et al. 2004).

The leafminer *C. triadicae* is not a classical biological control agent of tallow but was discovered in the tallow-invaded range during research conducted on the invasive weed. Due to distinctive morphological characteristics, *C. triadicae* is thought to have originated in Asia (Davis et al. 2013). Several unsuccessful attempts were made to find this species on tallow in China (G. S. W. unpublished data). At least 3 congeneric species were discovered feeding on tallow in its native range, causing the same distinct leaf damage. However, further analysis indicated they were not *C. triadicae* (Davis et al. 2013). Biological control efforts are currently focused on several species that show promise in terms of specificity and impact against tallow (Wheeler & Ding 2014). These include the flea beetle *Bikasha collaris* (Baly) (Coleoptera: Chrysomelidae) (Huang et al. 2011) and the defoliating caterpillar *Gadirtha fusca* Pogue (Lepidoptera: Nolidae) (Wang Y et al. 2012; Pogue 2014). Once released, biological control agents may come in contact with these *C. triadicae* parasitoids. However, it is unlikely that the niche-specific parasitoids found in this survey will attack these potential biological control agents as the larvae of *B. collaris* feed on tallow roots in soil and the *G. fusca* larvae are large (~350 mg dry weight) exposed foliage feeders. Considering the demonstrated niche specificity of these parasitoids, it is unlikely that they will attack the biological control agents being developed to control the invasive weed tallow.

**Table 1.** Parasitoids reared from larvae and pupae of the leafminer *Caloptilia triadicae* collected feeding on Chinese tallowtree during 2013–2014 in Florida.

Family	Subfamily	Genus	Species	Percentage of parasitoid complex
Bethylidae	Bethylinae	<i>Goniozus</i>	sp.	76
Eupelmidae	Eupelminae	<i>Brasema</i>	sp.	18
Eulophidae	Eulophine	<i>Sympiesis</i>	sp.	6
Eulophidae	Eulophine	<i>Zagrammosoma</i>	<i>multilineatum</i>	<1
Eulophidae	Eulophine	<i>Euplectrus</i> or <i>Platyplectrus</i>	sp.	<1





**Fig. 2.** Parasitoid species reared from larvae and pupae of *Caloptilia triadicae* feeding on Chinese tallow leaves in Florida. A. *Sympiesis* sp. female; B. *Sympiesis* sp. male (Hymenoptera: Eulophidae); C. *Brasema* sp. (Hymenoptera: Eupelmidae); D. *Goniozus* sp. (Hymenoptera: Bethyliidae); E. *Zagrammosoma multilineatum* (Hymenoptera: Eulophidae); F. *Euplectrus* sp. or *Platyplectrus* sp. (Hymenoptera: Eulophidae). Horizontal scale bars represent 1 mm in A, B, and C and 0.5 mm in D, E, and F.

## Acknowledgments

We acknowledge W. Pierre, J. Lollis, S. Steininger, C. Nguyen, J. Duncan, and K. Bowers (USDA/ARS), who provided technical assistance. *Caloptilia triadicae* specimens were generously identified by D. Davis, Smithsonian Institution, Washington, District of Columbia. Assistance with parasitoid identification was generously provided by M. Gates (USDA/ARS/SEL, Beltsville, Maryland), J. Heraty (U.C. Riverside, Riverside, California), G.A.P. Gibson (Agriculture and Agri-Food Canada, Ottawa, Canada), and C. Tribull (American Museum of Natural History). DNA barcoding analysis was conducted by R. Hazen, Tulane University (GenBank accession numbers KF061045–KF061062). This research was partially funded by the Florida Fish and Wildlife Commission, Florida Department of Environmental Protection (to G. S. W.) and USDA/ARS.

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