Host Range of Caloptilia triadicae (Lepidoptera: Gracillariidae): An Adventive Herbivore of Chinese Tallowtree (Malpighiales: Euphorbiaceae)

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Source: Florida Entomologist, 99(1) : 142-145

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.0132

Host range of *Caloptilia triadicae* (Lepidoptera: Gracillariidae): an adventive herbivore of Chinese tallowtree (Malpighiales: Euphorbiaceae)

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Chinese tallowtree, *Triadica sebifera* (L.) Small (Malpighiales: Euphorbiaceae), native to China, is one of the most aggressive and widespread invasive weeds in temperate forests and marshlands of the southeastern USA (Bruce et al. 1997). Chinese tallowtree (hereafter "tallow") was estimated to cover nearly 185,000 ha of southern forests (Invasive.org 2015). Since its introduction, the weed has been reported primarily in 10 states including North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, Texas, and California (EddMapS 2015). Tallow is now a prohibited noxious weed in Florida, Louisiana, Mississippi, and Texas (USDA/NRCS 2015). As the existing range of tallow is expected to increase, the projected timber loss, survey, and control costs will also increase. Cost estimates for controlling tallow infestations in forestlands of eastern Texas, Louisiana, and Mississippi range from $200 million to $400 million in the next 20 yr (Wang et al. 2012a). Tallow invades mesic flatwoods, scrubby flatwoods, alluvial floodplain forests, strand swamps, and ruderal communities. Chemical and mechanical control measures have been used with short-term success. However, long-term, cost-effective maintenance programs that integrate several control methods are required to prevent regrowth and recruitment (Jubinsky & Anderson 1996). Herbivory, through classical biological control, can provide an ecologically sound, cost-effective, and sustainable management solution to protect native plants in these habitats (Wheeler & Ding 2014).

One significant factor in the success of tallow in its invaded range is the historical lack of specialized herbivores that exert population-level regulation (Harcombe et al. 1993; Bruce et al. 1997). Herbivores in the USA that feed on tallow include mostly generalists, such as *Melanoplus angustipennis* (Dodge) and *Orphullela pelidna* Burmeister (Orthoptera: Acrididae) (Siemann & Rogers 2003; Lankau et al. 2004). The only herbivore with a narrow host range found feeding on tallow in its invaded range is the moth *Caloptilia triadicae* Davis (Lepidoptera: Gracillariidae). The early instars of this species mine the leaves, whereas the last instars cut out a leaf section and create a distinctive helical retreat where pupation occurs (Davis et al. 2013). This adventive species has now been reported from nearly all the tallow-infested areas of the southeastern USA (Fox et al. 2012). *Caloptilia triadicae* is thought to be of Chinese origin and was first discovered in the invaded range in 2004 (Fox et al. 2012).

Research on the biological control of tallow began in 2007 and identified a number of promising candidates from the weed's native range (Wheeler & Ding 2014). These candidates include the defoliating/root-feeding flea beetle *Bikasha collaris* (Baly) (Coleoptera: Chrysomelidae) and the defoliating moth *Gadirtha fusca* Pogue (Lepidoptera: Nolidae), both being tested in quarantine to determine suitability for biological control (Huang et al. 2011; Wang et al. 2012b; Pogue 2014). The compatibility of these potential agents with one another and other herbivores like *C. triadicae* is being examined. The goal of this study was to determine if *C. triadicae* posed a threat to other native or ornamental plants of the southeastern USA.

Plants. Tallow plant material was field collected as seeds, seedlings, or small plants in Alachua County, Florida, and cultured as potted plants and maintained in a secure area at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Additional plants were grown from seeds and seedlings provided by E. Siemann (Rice University, Houston, Texas). Non-target species were purchased or field collected (Table 1). Tallow plants and non-target species were maintained year-round inside a hoop house, 15 × 25 m, covered with woven shade cloth (30% black). The cloth only provided shade and allowed passage of *C. triadicae* moths into the hoop house. To protect plant species sensitive to cold temperatures between mid-Nov and mid-Apr, the hoop house was covered with 2 layers of clear 40 × 50 feet (12.19 × 15.24 m) 6 mL Super Dura-Film (At Films, Inc., Edmonton, Canada). Temperature was set at 29 °C and maintained by a thermostatically controlled gas heater (Modine Effiency II), a vent at the back (Micro-grow Greenhouse Systems), and a fan at the front of the hoop house. Tallow and non-target plants were intermingled and haphazardly arranged inside the hoop house.

Production and maintenance of plants was similar to that described in Steininger et al. (2013). Test plants were routinely sprayed with soap and water and fungicides as needed to exclude pests and pathogens. Fungicides included Cleary Chemical 3336WP Turf and Ornamental Systemic Fungicide (1.0 mL/L of water) and Spectracide Immunox Multi-Purpose Fungicide Spray Concentrate (7.8 mL/L of water). The plants were fertilized (Scotts Peters General Purpose Florida 20-20-20) approximately every 2 to 4 mo according to label rate. Additionally, 1 top coat of Osmocote Plus 15-9-12 controlled-release granular fertilizer was added to each potted plant (3.7 g/L). Plants were hand watered 1 to 3 times a week as needed and were maintained either outdoors or in the hoop house.

Insects. Populations of *C. triadicae* appear to be multivoltine during much of the year in Florida. Mines have been observed most often on new growth. Adults and larvae appear early in the spring (May) and disappear, possibly entering diapause, during the winter (Nov) as tallow loses its leaves.

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Table 1. List of tallow (in bold) and non-target plant species exposed to natural populations of the leaf miner *Caloptilia triadicae* in open-field multiple-choice tests. Plant phylogeny follows Wurdack et al. (2005), Wurdack & Davis (2009), and Govaerts et al. (2015).

Order/Family	Tribe	Species	Replicates	Complete development
Malpighiales				
Euphorbiaceae	Hippomaneae	***Triadica sebifera*** (L.) Small	47	+
		Ditrysinia (= *Sebastiania) fruticosa* (Bartram) Govaerts & Frodin	9	—[a]
		Gymnanthes lucida Sw.	8	+
		Hippomane mancinella L.	3	—[a]
		Neoshirakia japonicum (Siebold & Zucc.) Esser	4	—
		Stillingia sylvatica L.	6	—
	Euphorbieae	*Euphorbia* (= *Poinsettia*) *cyathophora* Murray	6	—
		Euphorbia (= *Poinsettia*) *heterophylla* L.	6	—
		Euphorbia (= *Chamaesyce*) *hirta* L.	12	—
		Euphorbia (= *Chamaesyce*) *hypericifolia* L.	6	—
		Euphorbia (= *Chamaesyce*) *hyssopifolia L.*	4	—
		Euphorbia (= *Chamaesyce*) *maculata* L.	6	—
		Euphorbia milii Des Moul.	4	—
		Euphorbia (= *Poinsettia*) *pulcherrima* Willd. ex Klotzsch	6	—
		Euphorbia (= *Pedilanthus*) *tithymaloides* L.	10	—
	Acalypheae	*Acalypha chamaedryfolia* (Lam.) Müll. Arg.	2	—
		Acalypha gracilens A. Gray	5	—
		Acalypha wilkesiana (= *amentacea* subsp. *wilkesiana*) Müll. Arg.	5	—
		Ricinus communis L.	6	—
	Plukenetieae	*Dalechampia scandens* L.	20	—
	Aleuritideae	*Vernicia* (=*Aleurites*) *fordii* (Hemsl.) Airy Shaw	6	—
	Codiaeae	*Codiaeum variegatum* 'Mamey' (L.) Rumph. ex A. Juss.	6	—
		Codiaeum variegatum 'Petra' (L.) Rumph. ex A. Juss.	6	—
	Crotoneae	*Croton alabamensis* E. A. Sm. ex Chapm.	5	—
		Croton glandulosus var. *glandulosus* L.	7	—
		Croton linearis Jacq.	10	—
		Croton punctatus Jacq.	3	—
	Jatropheae	*Jatropha curcas* L.	9	—
		Jatropha gossypiifolia L.	10	—
		Jatropha integerrima Jacq.	7	—
		Jatropha multifida L.	6	—
		Jatropha podagrica Hook.	3	—
	Manihoteae	*Cnidoscolus urens* (= *stimulosus*) (L.) Arthur	3	—
		Manihot esculenta Crantz	4	—
		Manihot grahamii Hook.	7	—
Phyllanthaceae	Phyllantheae	*Breynia disticha* J. R. Forst. & G. Forst.	10	—
		Phyllanthus acidus (L.) Skeels	11	—
		Phyllanthus amarus Schumach. & Thonn.	12	—
		Phyllanthus tenellus Roxb.	6	—
		Phyllanthus urinaria L.	7	—
	Bridelieae	*Heterosavia* (= *Savia*) *bahamensis* (Britton) Petra Hoffm.	10	—
Putranjivaceae	not applicable	*Drypetes diversifolia* Krug & Urb.	4	—

[a]Larval mines were found on these species, but they never matured to adult moths.

Host Range Tests. The target weed, *T. sebifera* is assigned to the Euphorbiaceae, in the order Malpighiales (Stevens 2015). We compiled a test plant list that included 41 species from the Euphorbiaceae family and relatives (Wurdack et al. 2005; Wurdack & Davis 2009; Govaerts et al. 2015). Members of the related families Phyllanthaceae and Putranjivaceae were also included (Stevens 2015). Host range tests were conducted with these closest relatives as they are considered most vulnerable to non-target damage and may be attacked by herbivores with narrow host ranges (Futuyma & Agrawal 2009). The list included native (e.g., *Ditrysinia fruticosa* [Bartram] Govaerts & Frodin, *Gymnanthes lucida* Sw.), ornamental (e.g., *Euphorbia pulcherrima* Willd. ex Klotzsch, *Acalypha wilkesiana* Müll. Arg.), and several threatened and endangered species (e.g., *Hippomane mancinella* L.*, Heterosavia bahamensis* [Britton] Petra Hoffm.). Replicates generally included 7 to 8 (range 2 to 47) plants for tallow and non-target species (Table 1). Plant sources followed those previously described (Steininger et al. 2013).

Multiple-choice tests were conducted inside the hoop house during 2010 and 2011 in Gainesville, Florida, where plants of the target weed were also present as a control. Plants were observed daily, and when larval leaf mines or rolls were found, the plant was caged to see if adult *C. triadicae* would emerge. Alternatively, leaf mines were reared in cups (30 mL) and observed for adult emergence. Additional field observations of ornamental *G. lucida* plants were conducted in 2012 at Alice Wainwright and Crandon parks, Miami-Dade County, Florida, and at the United States Department of Agriculture, Agricultural Research Service (USDA/ARS), Invasive Research Lab in Broward County,

Florida. Mature larvae were collected from flush growth and reared in the laboratory to determine if they could complete development on *G. lucida*. Adults that emerged were sent to a specialist (D. Davis, Smithsonian Institution, Washington, District of Columbia) who confirmed their identity.

During both 2010 and 2011, the *C. triadicae* infestation reached high densities in Gainesville, Florida, as 100% of the leaves were mined on *T. sebifera* saplings. Damage was so great that stem dieback was observed, and occasionally seedlings died from the impact of the larval feeding. The *C. triadicae* larvae fed and developed to adults on tallow and the non-target species *G. lucida* (Table 1). A few mines were found on *H. mancinella* and *D. fruticosa* but these larvae did not complete development. When leaf mines and rolls were found in 2010 on the non-target *G. lucida*, the plants were caged to see if adults would emerge, but none did. In 2011, all *G. lucida* plants (n = 8) were found damaged by *C. triadicae* larvae. One exuvia from an emerged *C. triadicae* adult was found in a *G. lucida* leaf roll suggesting that at least 1 individual completed development on *G. lucida*. In all, 14 *C. triadicae* pupae were found on *G. lucida* leaves and when caged, 2 adults, or 14% of the pupae, emerged. The suitability of *G. lucida* as a host of *C. triadicae* was confirmed as the native plant populations observed at Alice Wainwright Park in Miami-Dade County and the ornamental plantings at the USDA/ARS facility in Broward County were found infested with the moth.

Few herbivore species feed and develop on the invasive populations of tallow in its invaded range. Those that do feed on tallow are generalists that feed opportunistically while also damaging economically important species. Tallow plants grown in garden plots in Florida are routinely damaged by generalist larvae of *Spodoptera latifascia* Walker (Lepidoptera: Noctuidae) and adults of the invasive weevils *Myllocerus undatus* Marshall, *Pachnaeus litus* (Germar), and *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) (G. S. Wheeler, unpublished data). Tallow plants are visited by predators including *Crematogaster* species (Hymenoptera: Formicidae), possibly feeding on extrafloral nectar. The species *C. triadicae* is the first adventive moth with a narrow host range reported feeding on tallow in North America (Fox et al. 2012; Davis et al. 2013).

The distribution of the test plants that showed some degree of non-target damage has mostly little geographic overlap with that of tallow, with the exception of *D. fruticosa*. The native plants *G. lucida* and *H. mancinella* are only reported from the most southern counties of Florida (Miami-Dade and Monroe Counties) (Wunderlin & Hansen 2008). The species *D. fruticosa* occurs from north central Florida to east Texas (Wunderlin & Hansen 2008). In its invaded range, tallow is a temperate species, extending from North Carolina, along the Gulf States to east Texas (EddMapS 2015). In Florida, tallow occurs primarily north of Tampa, Orlando, and Daytona Beach (EddMapS 2015). However, ornamental plantings of *G. lucida* were found infested with the moth *C. triadicae* in sub-tropical Broward and Miami-Dade Counties. These infestations are over 400 km south of the major range of *C. triadicae*–infested tallow (Leesburg, Florida). Although *C. triadicae* appeared to primarily feed on *T. sebifera*, its ability to use *G. lucida* as a host is of concern. Although damage appeared to be minor on *G. lucida*, the geographic range of *C. triadicae* could expand to include the Caribbean. Caribbean countries do not have invasive populations of *T. sebifera*, but endemic members of the genus *Sapium*, another close relative of *T. sebifera*, occur in Puerto Rico (USDA/NRCS 2015). These species were not included in our host range tests, but if *C. triadicae* disperses to the Caribbean, these species could be exposed to this seemingly beneficial introduced species. Considering the host range of *C. triadicae*, it is doubtful a release permit would have been pursued as a classical biological control agent of tallow.

We wish to acknowledge J. Lollis (USDA/ARS), who first observed a *Caloptilia* leaf-roll in the USDA laboratory and together with J. Gibson-Weston and R. Weaver provided technical assistance. *Caloptilia triadicae* specimens were generously identified by D. Davis, Smithsonian Institution, Washington, District of Columbia. DNA barcoding analysis was conducted by R. Hazen, Tulane University, Louisiana (GenBank accessions KF061045–KF061062). This research was partially funded by the Florida Fish and Wildlife Commission, the Florida Department of Environmental Protection (to G. S. Wheeler), and the USDA/ARS.

Summary

An adventive herbivore of the invasive weed Chinese tallowtree (*Triadica sebifera* [L.] Small; Malpighiales: Euphorbiaceae) first appeared in North America in 2004. Identified as a *Caloptilia triadicae* Davis (Lepidoptera: Gracillariidae), this leaf miner was found damaging *T. sebifera* saplings. In Gainesville, Florida, we exposed naturalized populations of *C. triadicae* to Chinese tallowtree and related plant species in multiple-choice tests. Our findings indicated that the larvae of this species had a narrow host range as they readily fed and completed development on Chinese tallowtree leaves. The only exception to this specificity was larval feeding and development on the native oysterwood, *Gymnanthes lucida* Sw. (Malpighiales: Euphorbiaceae). Naturalized populations of *C. triadicae* were also found in Miami-Dade and Broward Counties, Florida, feeding and developing on ornamental populations of *G. lucida*.

Key Words: *Triadica sebifera*; Gracillariidae; biological control of weeds; multiple-choice test

Sumario

Un herbívoro adventivo de la maleza invasora Chinese tallowtree (*Triadica sebifera* [L.] Small; Malpighiales: Euphorbiaceae) apareció por primera vez en América del Norte en 2004. Identificado como *Caloptilia triadicae* Davis (Lepidoptera: Gracillariidae), este minador se encontró dañar *T. sebifera* plantones. En Gainesville, Florida, expusimos poblaciones naturalizadas de *C. triadicae* a Chinese tallowtree y especies de plantas relacionadas en las pruebas de selección múltiple. Nuestros resultados indican que las larvas de esta especie tenía un estrecho rango de huéspedes, muy ancho, ya que alimentaron y desarrollaron solamente en las hojas Chinese tallowtree. La única excepción a esta especificidad fue la alimentación y el desarrollo de las larvas en el oysterwood nativo, *Gymnanthes lucida* Sw. (Malpighiales: Euphorbiaceae). También se encontraron poblaciones naturalizadas de *C. triadicae* en los condados Miami-Dade y Broward, Florida, que se alimentaron y se desarrollaronsobre poblaciones ornamentales de *G. lucida*.

Palabras Clave: *Triadica sebifera*; Gracillariidae; control biológico de malezas; pruebas de selección múltiple

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