Quarantine host range and natural history of *Gadirtha fusca*, a potential biological control agent of Chinese tallowtree (*Triadica sebifera*) in North America

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Abstract

Classical biological control can provide an ecologically sound, cost-effective, and sustainable management solution to protect diverse habitats. These natural and managed ecosystems are being invaded and transformed by invasive species. Chinese tallowtree, *Triadica sebifera* (L.) Small (Euphorbiaceae), is one of the most damaging invasive weeds in the southeastern USA, impacting wetlands, forests, and natural areas. A defoliating moth, *Gadirtha fusca* Pogue (Lepidoptera: Nolidae), was discovered feeding on Chinese tallowtree leaves in the weed's native range and has been tested for its suitability as a biological control agent. Natural history studies of *G. fusca* indicated that the neonates have five instars and require 15.4 days to reach pupation. Complete development from egg hatch to adult emergence required 25.8 days. No differences were found between males and females in terms of life history and nutritional indices measured. Testing of the host range of *G. fusca* larvae was conducted with no-choice, dual-choice, and multigeneration tests and the results indicated that this species has a very narrow host range. No-choice experiments indicated that most larvae died in <3 days when fed each of the 78 non-target taxa; a similar duration as larvae fed only water. Although 81.6% of the neonates fed Chinese tallowtree survived to adult, the only survivors in no-choice tests were those fed the four non-target taxa, *Euphorbia hypericifolia* L., *Euphorbia hyssopifolia* L., *Euphorbia milii* Des Moul., or *Gymnanthes lucida* Sw. where 14.3% or less of the larvae fed and completed development. The results of dual-choice tests indicated that very little of each of these non-target taxa was eaten when given a choice with Chinese tallowtree. Furthermore, when neonates were reared for multiple generations on each non-target taxon, no more than two generations were completed when fed the non-target *G. lucida*, whereas the larvae were unable to complete more than one generation when fed the remaining non-targets. These tests indicate that although a small amount of feeding may occur in no-choice conditions on four species of non-targets, the larvae will not be able to maintain a population for more than two generations on any species except the target weed Chinese tallowtree. This species may play an important role and contribute to the integrated control of this invasive weed.

Introduction

Chinese tallow, *Triadica sebifera* (L.) (Euphorbiaceae), hereafter 'tallow', is one of the most damaging invasive weeds in the southeastern USA, impacting wetlands,

forests, and natural areas (Bruce et al., 1997; Pile et al., 2017). Tallow-invaded coastal tallgrass prairie and wetland communities are transformed into woodland thickets (Bruce et al., 1995; Neyland & Meyer, 1997; Wang et al., 2011). Historically, tallow has been distributed worldwide for many purposes and has become naturalized mostly in temperate areas of the USA (Pile et al., 2017). Tallow infests 185 000 ha of southern forests, stranded swamps, flatwoods, and ruderal communities where it has invaded

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areas of 10 states that border the Gulf of Mexico and California (Miller et al., 2010; Rawlins et al., 2018). Tallow is a prohibited noxious weed in Florida, Louisiana, Mississippi, and Texas (USDA/NRCS, 2018). The projected economic impact of this invasive weed over the next 20 years in forestlands of Texas, Louisiana, and Mississippi, in terms of survey, timber losses, and control costs, ranges from $200 million to $400 million (Wang et al., 2012a). Chemical and mechanical control measures have been used with short-term success. Permanent cost-effective maintenance programs that integrate several control methods are required to prevent regrowth and recruitment (Jubinsky & Anderson, 1996). Classical biological control can provide an ecologically sound, cost-effective, and sustainable management solution to protect these habitats (Wheeler & Ding, 2014).

The native range of tallow includes the parts of China and northern Vietnam (Bingtao & Esser, 2008). In China, tallow occurs mostly in provinces south of the Yellow River (Zheng et al., 2005). One factor contributing to 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). The implementation of classical biological control presents a potentially safe and cost-effective option that can be a component of an integrated pest management program. As tallow has been cultivated for centuries in China, many pests are known (Zheng et al., 2005). Three fungal pathogens and 115 species of arthropods have been reported to damage tallow and related members of the *Triadica* genus. Many of these species are generalist defoliators, but a few are specialists. These specialist species are candidates for biological control of tallow that can be screened for possible release in the USA. At least three species showed promise following tests conducted in China (Wang et al., 2009, 2012b; Huang et al., 2011).

Biological control screening of potential agents for tallow began in 2006 with foreign surveys initiated by the Wuhan Botanical Garden, Chinese Academy of Sciences (Beijing, China), in collaboration with USDA/ARS Invasive Plant Research Laboratory (IPRL, Ft Lauderdale, FL, USA). These surveys discovered several species of insects and preliminary testing was conducted on three: *Heterapoderopsis* (= *Apoderus*) *bicallosicollis* Voss (Coleoptera: Attelabidae), *Bikasha collaris* (Baly) (Coleoptera: Chrysomelidae), and *Gadirtha fusca* Pogue (Lepidoptera: Nolidae). These preliminary studies showed all three species had high specificity to the target weed (Wang et al., 2009, 2012b; Huang et al., 2011). Following these Chinese studies, all three species were imported and tested in quarantine at the USDA/ARS/IPRL facility. On testing North

American non-target species, the leaf-rolling weevil *H. bicallosicollis* was rejected due to broad host range (Steininger et al., 2013). Quarantine testing of the flea beetle *B. collaris* indicated that this species had a narrow host range and was well suited for biological control of tallow (Wheeler et al., 2017). Tests conducted on *G. fusca* in China indicated that this species was a potential biological control candidate (Wang et al., 2012b). These results lead to the current study that examined the host range of *G. fusca* in response to native and economic North American plant species.

The defoliator *G. fusca* is a multivoltine species found feeding on tallow leaves in Anhui, Guangdong, Guangxi, Hunan, and Jiangxi provinces of China (Wheeler et al., 2018). In preliminary field, host range tests conducted in China, 32 plant taxa from 17 plant families growing adjacent to *G. fusca*-infested tallow plants were checked visually (Wang et al., 2012b). These field surveys indicated that *G. fusca* individuals were only found on tallow. Additionally, laboratory tests examined the no-choice host range of *G. fusca* on 46 plant species from four families. The larvae completed the development only on the target weed *T. sebifera* and two other *Triadica* species from China (Wang et al., 2012b). Furthermore, the larvae were very damaging as six larvae per seedling caused 80% seedling mortality and reduced plant biomass by 60% (Wang et al., 2012b). These results indicate that *G. fusca* may be a safe and effective biological control agent for tallow. Our goal was to examine the host range of the defoliating caterpillar *G. fusca* in quarantine on plants relevant to its North American invaded range and to determine its suitability for field release as a classical biological control agent of tallow.

Materials and methods

Initial studies were conducted to describe the natural history of *G. fusca*. These studies were followed by host range tests to examine the potential of this species for biological control of tallow in the USA. Determination of the host range of *G. fusca* was examined by three testing protocols: no-choice, dual-choice, and multigeneration tests. This testing protocol followed that of another tallow biological control agent, *B. collaris* (Wheeler et al., 2017).

Tallow and non-target test plants

All plants were grown in greenhouse conditions during the fall and winter months and in an outside garden during the spring and summer at our facility at USDA/ARS/IPRL. All plants were grown using Pro-Mix PGX w/Biofungicide (germination mix) and Pro-Mix HP with Biofungicide and Mycorrhizae (Premier Tech, Quebec City, Canada).

Plants were fertilized at the labeled rate with Scotts Peter's Professional 20-20-20 Water Soluble Fertilizer, and Scotts Osmocote 15-9-12 Slow Release 3–4 months (Scotts, Marysville, OH, USA). To control pests, tallow plants were sprayed once a week with a liquid dish soap (Joy Lemon; Procter and Gamble, Cincinnati, OH, USA) solution (10 ml per 3.8 l water).

To predict the host range of *G. fusca*, a test plant list was compiled with the highest priority given to those species most closely related to tallow. However, plant species were also selected from diverse phylogenetic groups (Wheeler & Ding, 2014). The prioritization of plant species to be tested generally followed the phylogeny of the plant family, Euphorbiaceae to which tallow is assigned. Priorities were based on the centrifugal phylogenetic method recommended by Wapshere (1974) with modifications (Briese & Walker, 2008; Wheeler & Ding, 2014).

These test plant taxa were grouped into seven categories based on several criteria, including their phylogenetic relatedness to tallow, environmental, and recovery (e.g., endangered/threatened) status (TAG-BCAW-Manual, 2018). These include category 1: genetic types of the weed; category 2: species in the same genus; category 3: species in other genera in the same family; category 4: threatened and endangered species in the same family; category 5: species in other families in the same order; category 6: species in other orders; and category 7: any plant on which the proposed biological control agent or its close relatives have been found (TAG-BCAW-Manual, 2018). The test plant list for this target weed was compiled using the North American, Caribbean, and Mexican flora.

Organization of test plant taxa in these categories followed the phylogeny of the weed and its relatives. The taxa that are the close relatives of tallow are thought to be most vulnerable to non-target damage by biological control agents. Tallow is assigned to the large family Euphorbiaceae in the Malpighiales by the Angiosperm Phylogeny Group IV (APG IV, 2016). Other authorities place the Euphorbiaceae in its own order, Euphorbiales (USDA/ NRCS, 2018). The phylogeny follows that of Wurdack et al. (2005), Wurdack & Davis (2009), WCSP (2018), and Riina & Berry (2013). In the USA, there are 65 genera (including the genera of Phyllanthaceae and Putranjivaceae) in the family and 596 accepted taxa (USDA/NRCS, 2018). Included here are the genera of the now distinct families Phyllanthaceae and Putranjivaceae, as they were previously included in the Euphorbiaceae (APG IV, 2016). The family Euphorbiaceae is organized into four subfamilies, of which only Acalyphoideae, Crotonoideae, and Euphorbioideae occur in the invaded range of tallow (Wurdack et al., 2005; APG IV, 2016). The Euphorbioideae subfamily has

five tribes and 54 genera. In tallow's invasive range, only two tribes occur, the Hippomaneae and Euphorbieae. The tribe Hippomaneae contains a single subtribe, Hippomaninae, to which tallow is assigned. The tribe Euphorbieae also has a single subtribe in tallow's invaded range, Euphorbiinae. Thus, the taxa assigned to the tribe Hippomaneae received the greatest priority for testing. Although the susceptibility of these taxa was the focus of host testing, representatives distributed throughout the family were also tested.

The *Triadica* taxon is a small genus and is endemic to eastern and southeastern Asia (Esser, 2002). The genus is well circumscribed with only three accepted taxa and very probably monophyletic (Esser et al., 1997). Tallow was previously placed in the *Sapium* genus and upon revision reassigned to the Asian *Triadica* genus (Esser, 2002). No members of the *Triadica* genus are native to the New World. The closest relatives in North America are members of the same subtribe, Hippomaninae, which includes *Ditrysinia* (= *Sebastiania*) *fruticosa* (Bartram) Govaerts & Frodin, *Gymnanthes lucida* Sw., *Hippomane mancinella* L., *Sebastiania bilocularis* S. Watson, and *Stillingia sylvatica* L. (USDA/NRCS, 2018). Two Caribbean taxa assigned to this subtribe outside the invaded tallow range include *Sapium laurifolium* (A. Rich.) Griseb. and *Sapium laurocerasus* Desf. (USDA/NRCS, 2018). Another species from this genus, *Sapium glandulosum* (L.) Morong, was collected in Pensacola, Florida, in 1901 (NYBG, 2013). However, this species should not be treated as part of the USA flora as it is a single collection, a 'waif', that was never recollected after more than 100 years (P Berry, University of Michigan Herbarium, pers. comm.; Nelson, 2011). This species has also been reported from the Virgin Islands, Dominica, and tropical America (Wunderlin & Hansen, 2008; WCSP, 2018). Species of the Euphorbiaceae with some agricultural or ornamental significance include *Jatropha gossypiifolia* L. (bellyache bush), *Manihot esculenta* Crantz (cassava), and *Euphorbia* (= *Poinsettia*) *pulcherrima* Willid. Ex Klotzch (poinsettia).

Insect source

Chinese collections of *G. fusca* were initially imported in June 2012 to USDA/ARS/IPRL under quarantine. These larvae were collected feeding on tallow near Guilin, Guangxi, China. After colonization, larvae were examined for pathogens and after finding no disease, testing for specificity began. Additional Chinese introductions of field-collected larvae occurred in 2015 and 2016. The colonies were combined following DNA molecular examination that indicated all collections were the same taxon (Wheeler et al., 2018).

Colony maintenance and life-history tests

Routine *G. fusca* colony rearing of larvae and pupae was conducted with both solitary individuals (in 250-ml plastic container) and en masse (ca. 15 individuals in 1–l plastic container). Emerging adults were transferred to mating cages (61 × 61 × 91 cm; www.livemonarch.com) where adult male (n = ca. 10) and female (n = ca. 10) moths were provisioned with honey water (1:5) or Gatorade sports drink (lemon-lime flavor; PepsiCo, Purchase, NY, USA). Eggs were laid on strips of paper towel pinned to the interior of the cage. Previous research (GS Wheeler, unpubl.) indicated that females oviposit indiscriminately on all available surfaces. Paper strips containing eggs were moistened and transferred to a plastic container (1 l) with screen mesh on the lid to provide ventilation.

Naïve neonates were collected using a paintbrush and placed in 5.5-cm-diameter Petri dishes containing moistened filter paper. Tender apical tallow leaves were supplied to each larva. Larvae were checked daily to monitor feeding, molting, and survivorship. Leaves were changed every 1–3 days, frass was removed daily, and filter paper was moistened as needed. Molted head capsules of each instar were collected using a paintbrush and stored in Petri dishes. Head capsules were measured using a VHX 600-E Digital Microscope (± 0.1 μm; Keyence, Itasca, IL, USA). As larvae grew, they were removed from Petri dishes to Ziploc sandwich containers (11 × 11 × 6.5 cm) with lids vented with fine plastic mesh. Containers were lined with a moistened paper towel.

Leaf consumption by *G. fusca* larvae was estimated by comparing the leaf area before and after feeding with a flatbed Epson 3590 Photo scanner (Epson America, Long Beach, CA, USA). Leaf area was measured to estimate feeding by larvae from third instars to pupation and scans were analyzed with Adobe Photoshop (extended v.4.0; Adobe Systems, San Jose, CA, USA). Leaves were changed every 2–3 days and care was taken to always provide sufficient leaf material so larvae would not starve. Paired control leaves were scanned similar to adjust consumption for possible changes in leaf area. If a leaf was wilted, consumed, or in poor condition, it was exchanged for a fresh leaf. To calculate nutritional indices for third instars to pupae, leaf area consumed was converted to dry weights by weighing control leaves (n = 20) of known areas after they were dried for 2 days (60°C) (Slansky & Scriber, 1985). Insect frass produced by third instars to pupation was collected daily, dried at 60°C for 2 days, and weighed (± 0.1 mg, E10640 analytical balance; Ohaus, Parsippany, NJ, USA). Pupae were sexed when 3 days old by the observation of the genital pore position and observed for adult emergence. Pupal dry weights were obtained following oven drying at 60°C for 2 days. Data were collected on dry weight consumption from third instar to pupa (mg), development time from egg hatch to pupation and adult (days), and pupal dry weight (mg). Leaf and pupal dry weight estimates were used to calculate nutritional indices such as the approximate digestibility (AD) of ingested food (%), efficiency of conversion of ingested (ECI) food to insect biomass (%), and efficiency of conversion of digested (ECD) food to insect biomass (%).

Quarantine host range tests

Initially, quarantine no-choice tests were conducted as they are considered the most rigorous and conservative of test designs used to define a candidate's fundamental or physiological host range (Van Klinken, 2000; Schaffner, 2001). The primary criticism of these tests is that they are too conservative (Schaffner, 2001) and they provide results that potentially lead researchers to overlook candidates that would be safe to release (Cullen, 1990; Schaffner, 2001). We also conducted dual-choice tests on a subset of larvae as these tests complemented the previous no-choice test results. Dual-choice tests may better simulate more natural conditions (Harley, 1969) and be better predictors of risk than other testing methods (Cullen, 1990). Dual-choice tests were conducted on those plant taxa that were eaten by larvae or where prolonged larval longevity occurred in no-choice tests. The dual-choice tests conducted with *G. fusca* larvae were the 'normal' choice tests as they simultaneously exposed the target weed and a single test taxon (Schaffner, 2001). Furthermore, we conducted no-choice multigeneration tests on the same subset of taxa with naïve *G. fusca* larvae.

No-choice tests. Host range testing of *G. fusca* larvae was conducted using a test plant list that included 78 taxa mostly from the previous *B. collaris* testing protocol (Wheeler et al., 2017). Larval no-choice tests generally included at least 10 replicates (one larva per replicate) for the closest relatives (members of the Hippomaninae subtribe) and five replicates for the remaining non-target taxa. Additional replicates were also included on taxa that elicited feeding and development. These increased replicates were included to decrease the likelihood of false negatives on the most vulnerable taxa (Haines et al., 2013). For each test, naïve neonates were transferred individually to 2–3 apical test plant leaves inside a vented plastic container (6 × 6 × 10 cm) provisioned with a moist paper towel. Control larvae were treated identically but were fed tallow leaves in similar containers. If a larva on a non-target survived beyond the third instar, leaf consumption was measured as described above. If a larva fed on the non-target taxon and reached the pupal stage, it was weighed and sexed as described above. To ensure

validity of the results, control neonates were reared to adult regardless of whether the larvae fed the non-target survived. If either the control larva or control pupa died, the test was repeated.

Dual-choice tests. Dual-choice tests were conducted to assess *G. fusca* larval feeding preference when offered tallow leaves and leaves of one of the five non-target taxa on which a few larvae developed (from Results): *Euphorbia hypericifolia* L., *Euphorbia hyssopifolia* L., *Euphorbia milii* Des Moul. red and yellow variety, and *Gymnanthes lucida* Sw. Although no larval development occurred on the *E. milii* yellow variety in the no-choice tests, it was included as feeding occurred on the red variety of the same species. For each dual-choice test, neonates were reared under the same conditions described in the life-history methods, except that leaves of both tallow and one of the five non-target taxa were provided to each larva until pupation. The leaf area of tallow and each non-target species was matched as closely as possible within each dish. Replicates included five neonates per choice test reared individually in 9-cm-diameter Petri dishes lined with moistened filter paper. A midline was drawn using permanent marker on the lid of each dish; one half of each dish was labeled *Triadica* and the other with the name of the non-target being tested. A scanned leaf of each taxon was placed on its corresponding dish half. Data on the leaf area consumed by each caterpillar during instar three through to pupation was collected according to methods described in the life-history section.

Multigeneration tests. Multigeneration tests were conducted to determine whether *G. fusca* could sustain a population when fed each non-target taxon on which it successfully developed in the no-choice tests. Here, we determined whether neonates could feed and complete development to the adult stage, produce eggs, and continue for three generations fed only each of the non-targets. As before, we included a tallow control and each of the same five taxa: *E. hypericifolia*, *E. hyssopifolia*, *E. milii* (both red and yellow varieties), and *G. lucida*. Initially, 20 neonates from the colony were fed leaves of each plant taxon as described above. All available adults that emerged from these initial 20 larvae, from each non-target, were transferred to mating cages. Each mating cage was provided with a tallow plant or bouquet of the respective non-target species from which the larvae had fed. Once neonates emerged, the plant or bouquet was removed and replaced with fresh material. Plant material continued to be replaced every 5 days. Fecundity was estimated by counting the neonates on each plant 5 days after removal from the mating cage and summed over the life of the adults. From the total number of neonates produced on tallow, no more than 20 were reared to determine survival to the adult stage. All neonates fed the non-targets were included as there were always fewer than 20 available. Neonates were carefully transferred with a paintbrush into 5.5-cm-diameter Petri dishes containing moistened filter paper and a young leaf of tallow or a non-target taxon. Larvae were checked daily for survivorship, feeding, and molting. Leaves were changed as needed, generally every 1–3 days. Frass was removed and filter paper was replaced and moistened daily. Four replicates of each taxon were included.

Statistical analysis

Prior to statistical analysis of all data, residuals were checked for agreement with the assumptions of ANOVA and transformed as appropriate. Natural history results – including larval dry weight consumption, development time from egg hatch to pupation and adult, AD of ingested food (%), ECI food to insect biomass (%), ECD food to insect biomass (%), and pupal dry weight – were compared by sex of the individuals with a one-way ANOVA ($\alpha = 0.05$; SAS software 2010; SAS Institute, Cary, NC, USA). No-choice results, for longevity of larvae when fed non-targets, were analyzed with a one-way ANOVA, and means were compared with water-fed controls with a Dunnett test ($\alpha = 0.05$). No-choice results of taxa where larvae survived, including larval consumption, development time to the pupal stage, and pupal fresh weight, were analyzed with a one-way ANOVA. No-choice results, for comparing survival (%) to the adult stage for larvae fed non-targets or tallow leaves, were analyzed with a χ^2 test. Dual-choice test results for comparing area consumed of tallow with each non-target leaf were analyzed by individual one-way ANOVAs ($\alpha = 0.05$). Multiple generation results, for comparing larval survival (%) and number of neonates/female, were analyzed by repeated measures two-way ANOVAs for species and generations with interaction.

Results

Life-history tests

Complete *G. fusca* development for individuals fed tallow leaves from egg hatch to adult emergence required an average ($\pm$ SE) of 25.8 $\pm$ 0.1 days (Table 1). Egg hatch required 4 days, larval development to pupation required 15.4 $\pm$ 0.3 days, and pupa to adult required 10.8 $\pm$ 0.2 days (Figure 1). All larvae, regardless of sex, required five instars to reach the pupal stage. Each instar had distinct head capsule widths and increased by 1.61× for females and 1.65× for males, which is in general agreement with Dyar's rule (Dyar, 1890) (Table 1). The average

duration of each instar ranged from 2.1 to 3.2 days (Table 1). Total larval development time from eclosion to pupation did not differ significantly between sexes (Table 1). Pupal dry weights averaged 114.9 ± 3.7 mg and also did not differ significantly by sex (Table 1). Similar dry weight

Table 1 Natural history of *Gadirtha fusca* larvae

Instar	n	Development time (days)	Head capsule width (µm)
1	19	3.0 ± 0.1	359.1 ± 20.8
2	19	2.2 ± 0.1	576.6 ± 27.5
3	19	2.1 ± 0.1	903.8 ± 18.4
4	19	3.1 ± 0.2	1543.8 ± 29.9
5	19	3.2 ± 0.1	2587.3 ± 39.8

	n	Mean ± SE	F^1	d.f.	P
Total development time to pupa (days)	20	15.4 ± 0.3	0.2	1,17	>0.6
Total development time to adult (days)	20	25.8 ± 0.1	0.14	1,15	>0.7
Pupal dry weight (mg)	20	114.9 ± 3.7	0.8	1,18	>0.3
Consumption (mg dry weight)	19	741.0 ± 39.3	1.56	1,18	>0.2
AD (%)	19	38.8 ± 2.0	0.05	1,17	>0.8
ECI (%)	19	16.2 ± 1.0	0.25	1,17	>0.6
ECD (%)	19	44.9 ± 4.1	0.71	1,17	>0.4

Mean (± SE) instar duration (days), head capsule width (µm), total development time to pupation and to adult emergence (days), pupal dry weight (mg), dry weight consumption (mg), approximate digestibility (AD, %), efficiency of conversion of ingested food to insect biomass (ECI, %), and efficiency of conversion of digested food to biomass (ECD, %).
[1]ANOVA comparisons between males and females.

consumption (741.0 ± 39.3 mg), AD of their ingested food (38 ± 2.0%), ECI food to insect biomass (16.2 ± 1.0%), and ECD food to insect biomass (44.9 ± 4.1%) occurred for larvae of each sex (Table 1).

Quarantine host range tests

No-choice tests. The results of quarantine no-choice tests indicated that, when tested on 78 taxa, *G. fusca* larvae had a very narrow host range. When fed most non-target taxa, neonates died within (mean ± SE) 2.2 ± 0.1 days. This was similar to larvae provided only moisture which averaged 2.7 ± 0.2 (n = 11) days. For those larvae fed these non-targets, the time to death was always less than for larvae provided only water. Despite high mortality, complete larval development occurred, though with reduced survival, when fed four non-target taxa: *E. hypericifolia*, *E. hyssopifolia*, *E. milii* (red variety), and *G. lucida* (Table 2). Differences were found in larval % survival to the adult stage when fed different species (χ^2 = 114.78, d.f. = 4, P<0.0001). Larvae fed tallow leaves had 81.6% survival compared with 14.3% survival, or less, when fed the non-target taxa (Table 2). Only one larva survived on each of *E. hypericifolia*, *E. milii* (red variety), and *G. lucida*, which precluded statistical analysis of the consumption, development time, and pupal weight results. However, three larvae fed *E. hyssopifolia* survived to the adult stage and were compared statistically with those fed tallow (Table 2). Pupal weight was greater in tallow-fed larvae ($F_{1,56}$ = 17.21, P<0.0001) but neither consumption ($F_{1,18}$ = 0.43, P>0.5) nor development time ($F_{1,97}$ = 1.87, P>0.1) differed.

Dual-choice tests. Dual-choice test results indicated that when the larvae were given a choice, significantly less leaf
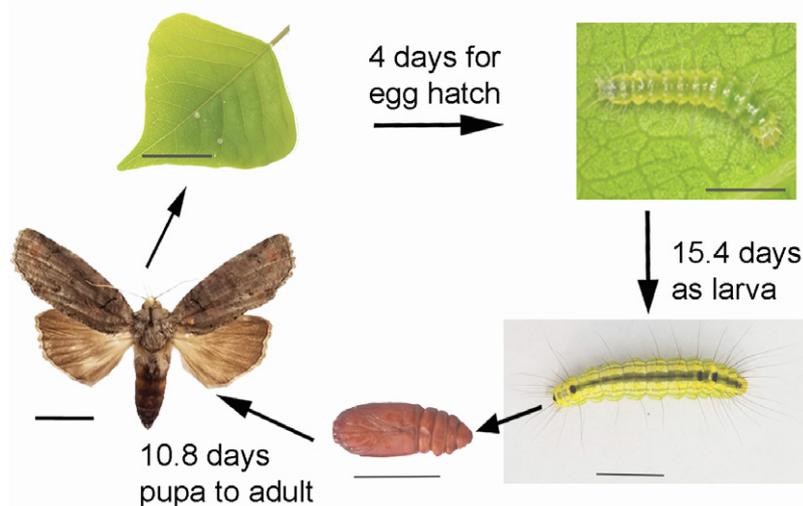
Figure 1 Life-history stages of *Gadirtha fusca* reared on leaves of *Triadica sebifera* in quarantine USDA/ARS/Invasive Plant Research Laboratory (Ft Lauderdale, FL, USA). Scale bar = 1 mm for small larva, 1 cm for all others.

4 days for egg hatch

15.4 days as larva

10.8 days pupa to adult

Table 2 Mean survival to adult (%), pupal weight (mg), consumption (mm^2), and development time to pupa (days) for *Gadirtha fusca* larvae when fed non-target or target (tallow) in no-choice tests

Species	n	% survival to adult	Pupal weight (mg)[1]	Consumption (mm^2)	Development time to pupa (days)
Category 1—Genetic types of tallow found in North America					
Malpighiales					
Euphorbiaceae					
Triadica sebifera (L.) Small	114	81.6	382.8a	278.6	18.7
Category 2—Species in the same (or closely related) genus as tallow, including environmentally and economically important					
Malpighiales					
Euphorbiaceae: subfamily Eurphorbioideae; tribe Hippomaneae; subtribe Hippomaninae					
Sapium glandulosum (L.) Morong	15	0			
Sapium laurifolium (A. Rich.) Griseb.	6	0			
Sapium laurocerasus Desf.	23	0			
Category 3—Species in other genera in the same family as tallow, divided by subfamily and tribes, including environmentally and economically important species					
Malpighiales					
Euphorbiaceae: subfamily Euphorbioideae; tribe Hippomaneae; subtribe Hippomaninae					
Ditrysinia (= *Sebastiania*) *fruticosa* (Bartram) Govaerts & Frodin	11	0			
Gymnanthes lucida Sw.	12	8.3	302.4	169.1	26.0
Sebastiania bilocularis S. Watson	12	0			
Stillingia sylvatica L.	10	0			
Euphorbiaceae: subfamily Euphorbioideae; tribe Euphorbieae; subtribe Euphorbiinae					
Euphorbia (= *Poinsettia*) *cyathophora* Murray	5	0.0			
Euphorbia graminea Jacq.	8	0			
Euphorbia (= *Poinsettia*) *heterophylla* L.	5	0			
Euphorbia (= *Chamaesyce*) *hirta* L.	10	0			
Euphorbia (= *Chamaesyce*) *hypericifolia* L.	15	6.7	393.7	233.2	23.0
Euphorbia (= *Chamaesyce*) *hyssopifolia* L.	21	14.3	197.4b	172.3	21.5
Euphorbia (= *Chamaesyce*) *maculata* L.	5	0			
Euphorbia milii Des Moul. (red variety)	11	9.1	396.0		27.0
Euphorbia milii Des Moul. (yellow variety)	16	0			
Euphorbia milii Des Moul. (unknown variety)	16	0			
Euphorbia (= *Chamaesyce*) *pinetorum* Small	8	0			
Euphorbia polyphylla Engelmann ex Chapman	2	0			
Euphorbia (= *Poinsettia*) *pulcherrima* Willd. ex Klotzch	5	0			
Euphorbia tirucalli L.	5	0			
Euphorbia (= *Pedilanthus*) *tithymaloides* L.	5	0			
Euphorbiaceae: subfamily Eurphorbioideae; tribe Hureae					
Hura crepitans L.	5	0			
Euphorbiaceae: subfamily Acalyphoideae; tribe Acalypheae; subtribe Acalyphinae					
Acalypha arvensis Poepp.	5	0			
Acalypha chamaedryfolia (Lam.) Mull. Arg.	5	0			
Acalypha gracilens A. Gray	5	0			
Acalypha (= *reptans*) *herzogiana* Pax & K. Hoffm.	5	0			
Acalypha ostryifolia Riddel ex J.J. Coult	5	0			
Acalypha wilkesiana (= *amentacea* subsp. *wilkesiana*) Mull. Arg	5	0			
Euphorbiaceae: subfamily Acalyphoideae; tribe Acalypheae; subtribe Ricininae					
Ricinus communis L.	5	0			
Euphorbiaceae: subfamily Acalyphoideae; tribe Acalypheae; subtribe Ricininae					
Caperonia castaneifolia (L.) A. St.-Hil.	5	0			

Table 2. Continued

Species	n	% survival to adult	Pupal weight (mg)[1]	Consumption (mm^2)	Development time to pupa (days)
Caperonia palustris(L.) A. St.-Hil.	6	0			
Euphorbiaceae: subfamily Acalyphoideae; tribe Plukenetieae; subtribe Dalechampiinae					
Dalechampia scandens L.	5	0			
Euphorbiaceae: subfamily Crotonoideae; tribe Aleuritideae; subtribe Aleuritinae					
Vernicia (= *Aleurites*) *fordii* (Hemsl.) Airy Shaw	5	0			
Euphorbiaceae: subfamily Crotonoideae; tribe Codiaeae					
Codiaeum variegatum 'Mammy' (L.) A. Juss.	5	0			
Codiaeum variegatum 'Petra' (L.) A. Juss.	5	0			
Euphorbiaceae: subfamily Crotonoideae; tribe Crotoneae					
Croton alabamensis E.A. Sm. ex Chapm	6	0			
Croton argyranthemus Michx.	5	0			
Croton glandulosus L.	5	0			
Croton linearis Jacq.	5	0			
Croton punctatus Jacq.	5	0			
Euphorbiaceae: subfamily Crotonoideae; tribe Jatropheae					
Jatropha curcas L.	5	0			
Jatropha gossypiifolia L.	6	0			
Jatropha integerrima Jacq.	5	0			
Jatropha multifida L.	5	0			
Jatropha podagrica Hook.	5	0			
Euphorbiaceae: subfamily Crotonoideae; tribe Manihoteae					
Cnidoscolus urens (= *stimulosus*) (L.) Arthur	5	0			
Manihot esculenta Crantz	5	0			
Manihot grahamii Hook.	5	0			
Category 4—Threatened and endangered species in the same family as tallow					
Hippomane mancinella L.	10	0			
Euphorbia telephioides Chapm.	5	0			
Ditaxis argothamnoides (= *Argythamnia blodgettii*) (Bertero. ex Spreng.) Radcl.-Sm. & Govaerts	3	0			
Tragia saxicola Small	5	0			
Croton humilis L.	5	0			
Manihot walkerae Croizat	5	0			
Heterosavia (= *Savia*) *bahamensis* (Britton) Petra Hoffm.	5	0			
Category 5—North American or introduced species in other families in the same order that have some phylogenetic, morphological, or biochemical similarities to tallow					
Phyllanthaceae: tribe Bischofieae					
Bischofia javanica Blume	5	0			
Phyllanthaceae: tribe Phyllantheae					
Breynia disticha J.R. Forst. & G. Forst.	5	0			
Phyllanthaceae: tribe Phyllantheae; Subtribe Flueggeinae					
Flueggea virosa (Roxb. ex Willd.) Royle	5	0			
Glochidion puberum (L.) Hutch.	5	0			
Phyllanthus acidus (L.) Skeels	6	0			
Phyllanthus pentaphyllus C. Wright ex Griseb.	5	0			
Phyllanthus tenellus Roxb.	5	0			
Phyllanthus urinaria L.	5	0			
Phyllanthaceae: tribe Poranthereae					
Phyllanthopsis (= *Leptopus*) *phyllanthoides* (Nutt.) Voronts. & Petra Hoffm.	5	0			

Table 2. Continued

| Species	n	% survival to adult	Pupal weight (mg)[1]	Consumption (mm^2)	Development time to pupa (days)
Putranjivaceae					
Drypetes lateriflora (Sw.) Krug & Urb	5	0			
Category 6—North American or introduced species in other orders that have some phylogenetic, morphological, or biochemical similarities to tallow					
Rosales					
Rosaceae					
Prunus caroliniana Aiton	6	0			
Eriobotrya japonica (Thunb.) Lindl	5	0			
Sapindales					
Rutaceae					
Citrus × *aurantium* L.	5	0			
Citrus jambhiri Lush.	5	0			
Zanthoxylum faga (L.) Sarg.	5	0			
Myricales					
Myricaceae					
Morella (= *Myrica*) *cerifera* (L.) Small	12	0			
Cyperales					
Poaceae					
Saccharum officinarum L.	5	0			
Lamiales					
Verbenaceae					
Vitis rotundifolia Michx.	5	0			
Myrtales					
Lythraceae					
Lagerstroemia indica L.	6	0			
Lagerstroemia (*indica* × *fauriei*) 'Natchez'					
Buxales					
Buxaceae					
Pachysandra procumbens Michx.	6	0			
Illiciales					
Illiciaceae					
Illicium parviflorum Michx. ex Vent.	7	0			
Caryophyllales					
Cactaceae					
Consolea (= *Opuntia*) *corallicola* Small	4	0			

Each replicate represents a neonate fed excised leaves.

[1]Comparisons limited to those means that had >1 value (i.e., *T. sebifera* and *E. hyssopifolia* only). Means followed by different letters are significantly different (one-way ANOVA: <0.05).

area was consumed of each non-target compared with the tallow leaves (Figure 2). In a few non-target taxa, a small amount of leaf damage was noted; however, this nibbling always amounted to <5% of the damage to tallow leaves.

Multigeneration tests. The *G. fusca* larvae survived and reproduced for three generations on tallow leaves. When offered leaves of *G. lucida*, the larvae only completed two generations, whereas larvae offered leaves of the remaining non-target taxa failed to develop beyond the first generation (Figure 3A). When fed tallow leaves, over 92% of the larvae completed development to the adult stage during all three generations. By comparison, 12.5 and 15% of the larvae survived when fed *G. lucida* leaves during the first and second generations, respectively, but no larvae survived on this species to produce a third generation. For larvae fed the remaining non-target taxa, 0–7.5% survived during the first generation, but none
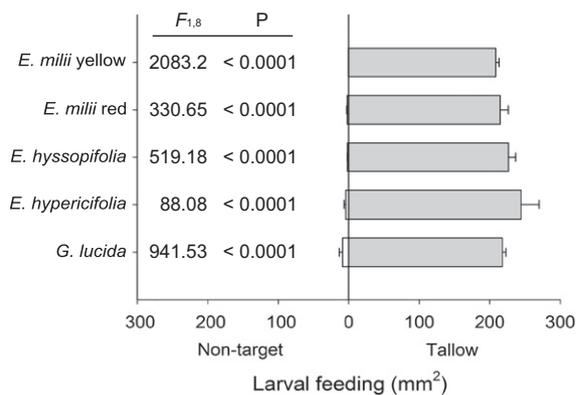
Figure 2 Mean (+ SE) larval consumption (mm²) for dual-choice tests where *Gadirtha fusca* larvae was offered a choice between tallow or non-target leaves. Plant species include *Euphorbia hypericifolia*, *E. hyssopifolia*, *E. milii* (yellow and red varieties), and *Gymnanthes lucida*.
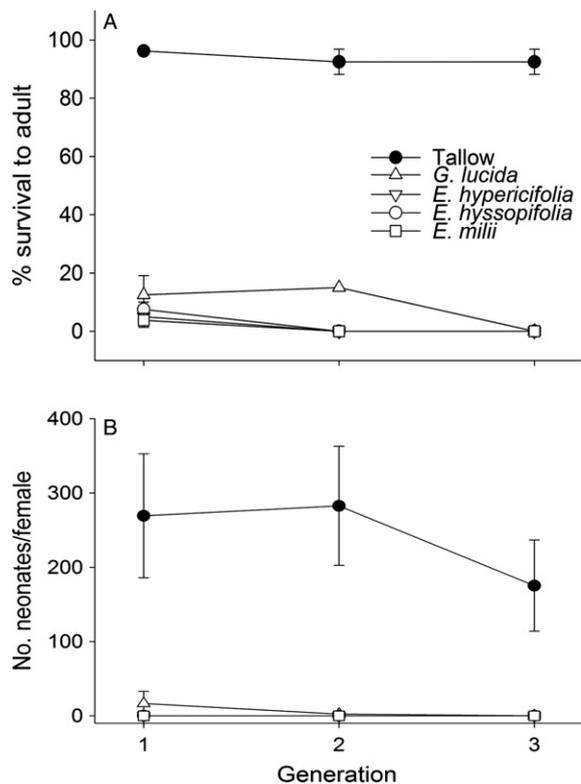
Figure 3 Mean (± SE) (A) survival (%) of neonates to adult and (B) number of neonates per female in multiple generation tests where *Gadirtha fusca* larvae were offered either tallow or non-target leaves. Plant species include *Euphorbia hypericifolia*, *E. hyssopifolia*, *E. milii* (yellow and red varieties combined), and *Gymnanthes lucida*.

achieved a second generation (Figure 3A). Survival of larvae fed the two varieties of *E. milii* was not different and they were combined (Figure 3A). Percent survival for these species was different ($F_{5,18}$ = 118.98, P<0.0001), whereas generations ($F_{2,6}$ = 0.09, P>0.9) and the species*generations interaction were not ($F_{1,6}$ = 0.4, P>0.5).

The plant species fed to the *G. fusca* larvae had a significant effect on the number of neonates produced per female ($F_{2,29}$ = 11.56, P = 0.0002). Adults that emerged from larvae fed tallow leaves produced over 250 neonates per female during the first and second generations, and 175 during the third generation (Figure 3B). By comparison, adults that emerged from larvae fed *G. lucida* leaves during the first and second generations produced 16.5 and 2.3 neonates per female, respectively. No neonates were produced by adults that emerged from larvae fed the remaining non-target taxa. The effect of the two varieties of *E. milii* on the neonates was not different and they were combined (Figure 3B). The number of neonates per female was also influenced by the generation of *G. fusca* ($F_{4,15}$ = 10.12, P = 0.0004) and a significant species*generation interaction ($F_{8,29}$ = 6.77, P<0.0001) which can be explained by the decrease in the number of neonates per female produced during the third generation in individuals fed tallow (Figure 3B).

Discussion

In total, 78 plant taxa were tested by no-choice in quarantine to determine the host range of *G. fusca*. The plant taxa included eight species from Hippomaninae, the subtribe that includes tallow, and 24 taxa from the Euphorbioideae, the subfamily to which the Hippomaninae belong. Moreover, we tested numerous taxa from the other two major Euphorbiaceae subfamilies, Acalyphoideae and Crotonoideae. Numerous non-target taxa from other plant families were also tested. In larval no-choice tests, *G. fusca* demonstrated a high degree of specificity toward the target weed, tallow. Complete larval development occurred but with reduced survival when fed four non-target taxa: *E. hypericifolia*, *E. hyssopifolia*, *E. milii* (red variety), and *G. lucida*. Although larvae had 81.6% survival when fed tallow, they had 14.3% or less survival when fed the non-target leaves. When *G. fusca* neonates were given a choice between tallow or each of these non-targets, they ate significantly more tallow than any non-target. Finally, multigeneration studies indicated that the larvae are not able to complete more than two generations on the non-target *G. lucida* and at most one generation on the remaining

non-targets. Should this insect be released a small number of larvae may complete development on these non-targets, but *G. fusca* will not be able to sustain a population on any species except tallow. Where tallow grows adjacent to these non-targets, the larvae will choose and feed on tallow in nearly all cases. These results confirm the preliminary specificity results from laboratory host range tests conducted in China (Wang et al., 2012b). This species and the flea beetle *B. collaris* may play an important role and contribute to the biological control of this invasive weed.

The most damaged non-target species by *G. fusca* was *G. lucida*, a close relative of tallow in the same subtribe Hippomaninae, and three species of *Euphorbia*, *E. hypericifolia*, *E. hyssopifolia*, and *E. milii* all members of the Euphorbiinae subtribe. The close tallow relative *G. lucida* occurs naturally in south Florida and the Caribbean but is more than 300 km distant from the invaded range of tallow (Rawlins et al., 2018; USDA/NRCS, 2018). The native species *E. hypericifolia* and *E. hyssopifolia* are naturally distributed through much of the tallow-invaded range and could co-occur with any biological control agents released against this invasive weed. Our results from dual-choice tests clearly show that when given a choice, these larvae will prefer the weed tallow over these *Euphorbia* species. Where a choice between these species is unavailable and the larvae are forced to feed on these native species or starve, a small amount of feeding may occur resulting in very low larval survival to the adult stage. Moreover, populations that are forced to establish on these *Euphorbia* species will not be sustained for more than one generation. The final species *E. milii* is an ornamental that does not overlap with tallow with a distribution that appears restricted to a few counties of south Florida.

Although these results show that the fundamental host range of *G. fusca* is highly conserved, the potential exists for rapid evolution of the specificity of this herbivore species (Futuyma, 2000; Hufbauer & Roderick, 2005). Despite the limited genetic diversity of the quarantine population tested and the expected low selection pressure post release, evolution of the fundamental host range may occur (Van Klinken & Edwards, 2002). However, historic reviews of weed biological control species released indicated that there is no convincing evidence that evolution of host range has occurred post introduction (Pemberton, 2000; Van Klinken & Edwards, 2002). Although of considerable concern, these reviews indicate that the host range of weed biological control agents are conserved, predictable, and stable.

Tallow is the most damaging non-native tree species in the southern USA ecosystems as it aggressively transforms invaded communities (Pile et al., 2017). This invasive species quickly invades diverse habitats due to a general lack of herbivore pressure, rapid growth, and high propagule pressure (Bruce et al., 1997; Pile et al., 2017). The research presented here seeks to develop permanent, cost-effective control methods that can be integrated with other management techniques. The Technical Advisory Group (USDA/APHIS, 2018) has reviewed and recommended the first tallow biological control agent for field release. If this agent and the defoliating *G. fusca* are approved for field release, they could have a significant impact on tallow populations and contribute to the integrated control of this invasive species.

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