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Pre-release assessment of *Gadirtha inexacta*, a proposed biological control agent of Chinese tallow (*Triadica sebifera*) in the United States

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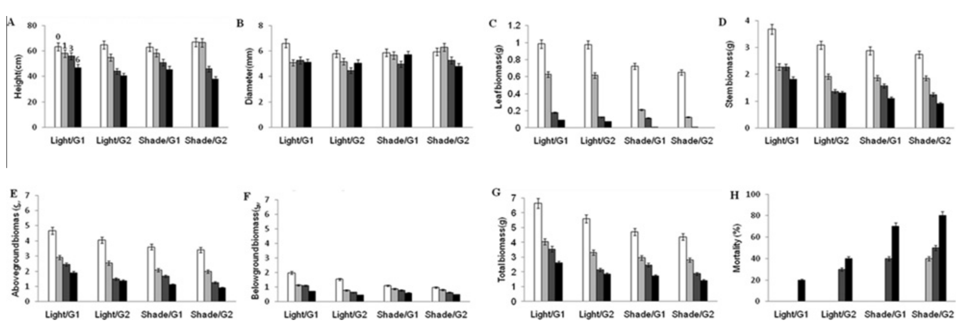
HIGHLIGHTS

- *Gadirtha inexacta* has a narrow host range.
- Attack by *G. inexacta* larvae significantly reduced the growth of *Triadica sebifera*.
- The impact was greater when the plant was repeatedly defoliated.
- The impact was greater in shade condition.

GRAPHICAL ABSTRACT



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ABSTRACT

Native to China, Chinese tallow, *Triadica sebifera* (Euphorbiaceae) is an aggressive woody invader in the southeastern United States. The noctuid, *Gadirtha inexacta* (=*Iscadia inexacta*), is a multivoltine herbivore attacking this plant in China. To evaluate its potential as a biological control agent in the United States, we conducted experiments on the host-specificity and impact on the target weed in China. The host range was tested on 78 plant species in 21 families through no-choice larval development tests and field surveys. The results showed that *G. inexacta* could only complete development on *T. sebifera*, *T. rotundifolia* and *T. chihsinianum*, suggesting a narrow host range. Attack by *G. inexacta* larvae significantly reduced the growth of seedlings of *T. sebifera* and the impact was greater when the plant was repeatedly defoliated and in shaded conditions. The plants were entirely defoliated after being repeatedly damaged in the treatments of the highest larval density (six larvae per seedling) in shade, causing 80% seedling mortality and the total biomass was reduced to 60% of the control. The results of this study suggested that *G. inexacta* may be a suitable biological control agent of *T. sebifera* though further tests on native North American species are needed.

1. Introduction

Pre-release studies on an invasive plant's natural enemies in the native range have long been considered effective means to screen potential biological control agents (McClay and Balciunas, 2005). Such assessments should include host-specificity tests of natural enemies and an evaluation of their impact on plant growth and reproduction, thereby providing predictions of safety, abundance and efficacy of potential agents in their new environment (Goolsby et al., 2006; Sheppard et al., 2006; Wang et al., 2008). Conducting such studies in the native range may be more convenient and

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efficient and allows for studies that are not possible in the introduced range. For example, open-field tests that are used to show an insect's ecological (field) host range can only be done in the native range (Briese et al., 1995; Briese and Walker, 2002).

Many factors affect insect feeding on plants, leading to either success or failure in biological weed control. For example, light levels may affect the plant's compensatory abilities (Harris, 1981; Trumble et al., 1993; Strauss and Agrawal, 1999). Milbrath (2008) reported that defoliation may be effective against invasive *Vincetoxicum rossicum* (Kleopow) Barbar. (Apocynaceae) and *Veratrum nigrum* (L.) Moench (Apocynaceae) plants growing in low light environments such as forest understories but appears to be less effective in high light environments unless repeated defoliation occurs. Wang et al. (2011) found that the leaf-rolling weevil, *Heterapoderopsis bicallosicollis* Voss (Coleoptera: Attelabidae) had larger populations on its host plant, Chinese tallow (or tallow), *Triadica sebifera* L. Small (synonyms: *Sapium sebiferum* L. Roxb.), in light than in shade conditions. Therefore, it is critical to understand how biological control agents affect plant performance along light gradients for tree species such as *Triadica*, which are both grassland and forest invaders.

Recent studies also show that plant tolerance to herbivory may affect the efficacy of biological control agents of invasive plants, as many invaders are able to tolerate herbivory by insects due to their high growth rate. In our previous study, we compared the tolerance to two leaf chewing herbivores between different populations of *Triadica* and found that introduced *Triadica* populations grew larger than those from native populations in all conditions (Wang et al., 2011) which suggest that increased tolerance to herbivory in introduced populations may impede success of biological control programs. However, in pre-release evaluations in biological control programs, very few studies have attempted to forecast the impact of the agents by examining tolerance (Hinz and Schwarzlaender, 2004; Morin et al., 2009). Many biological control agents have more than one generation per year allowing repeated defoliation which may enhance the impact of herbivory and counter plant tolerance. For example, Striker et al. (2011) reported that increasing frequency of defoliation constrained the regrowth of forage legume, *Lotus tenuis* Waldst. & Kit. However, impact studies frequently only evaluate the effect of insect damage within a single generation (Gerber et al., 2008; Huang et al., 2011; Smith, 2005), possibly underestimating the role of the herbivore.

Chinese tallow is a deciduous tree that originates from central and southern China (Zheng et al., 2005). Since it was introduced into the United States for agriculture in the late 18th century, Chinese tallow has become invasive in many parts of the southeastern United States (Bruce et al., 1997). It is listed as a noxious invasive weed in Florida, Louisiana, Mississippi, and Texas (USDA/NRCS, 2012) for displacing native plants and forming monospecific stands (Jubinsky and Anderson, 1996; Siemann and Rogers, 2003). Current management tactics, such as chemical and mechanical controls, are not long-term effective solutions considering that Chinese tallow grows rapidly and produces a large number of seeds annually (Siemann and Rogers, 2003). Biological control may be a permanent and cost-effective alternative means of Chinese tallow suppression. A positive aspect of investigating biological control is that Chinese tallow is the only species in the genus *Triadica* in the United States (Pemberton, 2000; Ding et al., 2006). Currently two insects, a flea beetle, *Bikasha collaris* (Baly) (Coleoptera: Chrysomelidae), and a noctuid moth, *Gadirtha inexacta* Walker (Lepidoptera: Noctuidae), are under evaluation for their potential as biological control agents. Here we report our pre-release assessment on *G. inexacta* in China.

The aims of this study were: (1) to evaluate the impact of *G. inexacta* on the growth of Chinese tallow and (2) to examine the host-specificity of *G. inexacta*. Since Chinese tallow invades both forest and grassland, we conducted experiments under different light conditions to predict the impact in these different habitats. In addition, previous studies show that Chinese tallow is able to tolerate herbivory by *G. inexacta* caterpillars (Huang et al., 2010; Wang et al., 2011) and as this insect has multiple generations per year in China, we designed a repeat defoliation experiment to mimic its effect in the field.

2. Materials and methods

2.1. Study organisms

In China *T. sebifera* seedlings appear in late April or May and become mature (set seeds) after 3–4 years and grow rapidly (50–70 cm in height per year). The height of mature trees ranges from 7–20 m (Zhang and Lin, 1994; Meyers, 2011; Zheng et al., 2005). Flowering occurs from July to August and seeds mature in late September. All *T. sebifera* plants used in our experiment were cultured from seeds collected from Luotian County, approximately 260 km east of Wuhan, Hubei Province, China.

Gadirtha inexacta overwinter as eggs, on branches and leaves, that hatch in May. The larvae, which develop through six instars in about 15 days, feed on leaves and can cause extensive defoliation, especially during the last three instars. The moth has 4–5 generations per year in the Hubei Province.

2.2. Impact experiment

From July to October 2008, we conducted an experiment to investigate the impact of *G. inexacta* on *T. sebifera* in the Wuhan Botanical Garden, at the Chinese Academy of Sciences, Hubei, China (30°32′ N, 114°24′ E). The weather in Wuhan at that time of the year is hot (average daily temperature range is from 25 to 35 °C) and rainy. Tallow tolerates a range of full sun to deep shade conditions (Jones and McLeod, 1989, 1990). To examine the effect of light on impact, caged plants were either exposed to ambient light (light treatment) or partially shaded with black polypropylene shade cloth (shade treatment). The nylon cage intercepted 30% of photosynthetic-active radiation measured weekly at midday with a PAR ceptometer (GLZ-A, Tuopu Ltd., Hangzhou, China), resulting in the plants receiving 70% ambient light. A deeper level of shade treatment was achieved using a layer of black shade cloth cover over the nylon cage which intercepted 88% of the photosynthetic-active radiation.

On 20 June 2008, similar-sized seedlings were selected for the experiments and transplanted individually into pots (height: 16 cm, diameter: 25 cm) containing growing medium (50% field soil and 50% sphagnum peat moss) and arranged in an outdoor common garden (distance between each pot is 50 cm). The seedlings were randomly assigned to different treatments. In the common garden each plant was enclosed by a nylon cage (100 cm height; 27 cm diameter) to exclude herbivores. Seedlings were tested because a previous study indicated that the early-seedling stage plays an important role in the invasion success of *T. sebifera* (Bruce et al., 1997).

On 21 July, third-instar larvae at one of four densities (0, 1, 3, 6 larvae/plant) were released onto each potted seedling. The survival of larvae was monitored and those that died during the course of the experiment were replaced with the corresponding larval stage. When larvae developed to pupae, they were removed. On 20 August, half of the potted plants received additional third-instar larvae to represent a second generation in the field which were released at the same densities as the first generation treatment (0, 1, 3, 6 larvae/plant). The other half of the plants had no added insects. Pots were randomly assigned to one of two light treatments, each group having either one or two generations of larvae.