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Epiblema tetragonana and *Epinotia ustulana* (Lepidoptera: Tortricidae), two potential biological control agents for the invasive plant, *Rubus ellipticus*

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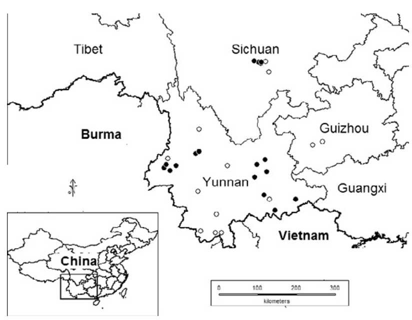
HIGHLIGHTS

- Consideration of leaf-rolling moths as biocontrol agents for *Rubus ellipticus*.
- Larvae of both moth species developed on plants in the genus *Rubus*.
- Both moth adults showed a strong preference for *R. ellipticus* over other species.
- Leaf-rolls by both species were only found on *R. ellipticus* in the field.
- Both are widely distributed and abundant in southwestern China.

GRAPHICAL ABSTRACT

Field survey sites in Southwestern China. Dots indicate where *Rubus ellipticus* and *Epiblema tetragonana* and *Epinotia ustulana* were found, circles indicate where only *R. ellipticus* but no *E. tetragonana* and *E. ustulana* were found.



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ABSTRACT

Himalayan yellow raspberry, *Rubus ellipticus* is one of the world's 100 worst invasive alien species. The plant has become a serious problematic weed in Hawaii, USA and is naturalized in many other countries. Screening of potential biological control agents is being conducted in its native region in Asia. In this paper, we report on the field distribution, abundance and host specificity of two leaf-rolling moth species, *Epinotia ustulana* and *Epiblema tetragonana* (Lepidoptera: Tortricidae). In larval non-choice tests both species only developed on plants in the genus *Rubus*. However, in adult choice oviposition tests, both leaf rollers showed a strong preference for *R. ellipticus* over other species. Furthermore, leaf-rolls by these two insects were only found on *R. ellipticus* in the field. These results indicate the moths have a narrow host range. Our field surveys also showed that both moth species are widely distributed in Yunnan Province, southeastern China, with up to 115 leaf rolls per plant, suggesting high levels of damage. These findings indicate that the two insects have considerable potential for biological control of *R. ellipticus*, though further host range tests should be conducted using more native plant species in Hawaii.

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1. Introduction

The Himalayan yellow raspberry, *Rubus ellipticus* (Rosaceae) is a highly invasive plant in Hawaii, USA and has naturalized in tropical Africa, tropical Southern America, the West Indies, and England (Evans et al., 2007; US Forest Service, 2010) and is considered one of the world's 100 worst invasive alien species (Global Invasive Species Database, 2006; Lu and Boufford, 2003; US Forest Service, 2010). The plant is native to Southeast Asia. It grows to 1–3 m and forms thickets of thorny shrub, with white or pink petals. Flowering occurs during March to April and large amounts of golden yellow aggregate fruits are produced from April to May (Lu and Boufford, 2003). It grows on slopes, in mountain valleys, sparse forests, thickets, and roadsides at elevations between 300 and 2600 m (Stratton, 1996). The plant has some economic value as it has been used as a traditional medical herb in its native range and the fruits are edible (US Forest Service, 2010).

R. ellipticus was introduced to Hawaii in 1961 and has since replaced native species in the resident plant communities rapidly (Jacobi and Warshauer, 1992; Lowe et al., 2000; Stratton, 1996). *R. ellipticus* has been considered a major threat to the Ola'a Forest Tract of Hawai'i Volcanoes National Park. The plant forms impenetrable thickets thereby threatening native lowland wet forests and displacing native plant species, including the native Hawaiian raspberry *Rubus hawaiiensis* (Stratton, 1996). In Australia, it is confined to the coastal strip of southern Queensland from Wide Bay to northern New South Wales (NSW), and there are some specimens from the central coast region of NSW (Evans et al., 2007). First reports of it being naturalized in Queensland were in 1912 where it has since been declared noxious (Evans et al., 2007).

Several methods, including chemical herbicides, physical and manual removal have been used for more than 20 years in attempts to control *R. ellipticus* in Hawaii, but these efforts have largely failed to reduce infestations (Markin et al., 1992; Santos et al., 1991; Santos et al., 1992). To control its closely related species, *Rubus argutus,* 3 months, *Schreckensteinia festaliella* Hubner (Lepidoptera: Helionidae), *Croesia zimmermani* Clarke (Lepidoptera: Tortricidae) and *Bembecia marginata* Harris (Lepidoptera: Aegeridae), one leaf beetle, *Chlamisus gibbosa* F. (Coleoptera: Chrysomelidae) and one sawfly, *Priophorus morio* Lepeletier (Hymenoptera: Tenthredinidae) were released in Hawaii (Nagata and Markin, 1986; Pemberton, 2002). Although *S. festaliella*, *C. zimmermani* and *P. morio* became established, there was no significant impact on either *R. argutus* or *R. ellipticus* (Gardner and Davis, 1982; Goeden et al., 1974). A biological control program was initiated against *R. ellipticus* in the 1990s, when Gardner (1999) carried out field inventories for potential insect and pathogen agents of *R. ellipticus* in its native region. No insects or pathogens have so far been released as agents.

Studies on insect host specificity, distribution and abundance in the native range of invasive plants are necessary for a successful biological control program, because these can indicate the potential safety and efficacy of the insects in the new environment (Goolsby et al., 2006; Wang et al., 2009). During 2006–2010, intensive faunal surveys were conducted and discovered 62 insect species in 22 families that predated on *R. ellipticus* in south-western China (Wu et al., 2013), of which, two leaf-rolling moth species, *Epinotia ustulana* and *Epiblema tetragonana* (Lepidoptera: Tortricidae) were found to be widely distributed in Yunnan Province, causing high levels of damage to the plants. To evaluate the two leaf-rollers' potential for biological control, we conducted field and laboratory tests in their host range. We also did extensive field surveys for their distribution and abundance under natural conditions.

2. Materials and methods

2.1. Study organisms

Female adults of *E. tetragonana* and *E. ustulanan* lay eggs on *R. ellipticus* leaves. Larvae roll and feed on leaves but also damage young shoots. There are generally five generations of *E. tetragonana* and three generations of *E. ustulanan* per year in Yunnan province. Larvae of both species occur on the plants from April to October. Because the two moth species share the same host and their leaf rolls are very similar in shape and size, it is hard to distinguish them in the field and only the pupae and adults can be identified (Wu et al., 2013). Larvae of both species in mixed batches were collected in Xundian County, Yunnan province China (N25°22′, E103°15′), from April 2008 to August 2009. The larvae were transported to our laboratory in Wuhan for rearing to adults, which were then separated into two groups whose off-spring were used for host range tests.

2.2. Laboratory host range tests

2.2.1. Plant species tested

Thirty species in seven families were selected for tests, based on their phylogenic relationship, biogeographic overlap and ecological similarity with *R. ellipticus* (Howarth et al., 1997; Pemberton, 2002) and plant availability. We also considered some economic and ecological species existing in Hawaii, according to Starr Environmental-Botanical and Faunal Surveys in the State of Hawaii, such as *Buxus microphylla, Cucurbita moschata, Morus alba, Duchesnea indica, Pyracantha fortuneana, Rosa chinensis, Sanguisorba officinalis, Eriobotrya japonica and Rubus rosifolius*. Plants were grown from seeds or transplanted from fields and were potted in under greenhouse conditions of 20–30 °C, 60–90% RH and a 14-h photoperiod. All the following experiments were carried out under the above environmental conditions.

2.2.2. Larval non-choice tests

To examine larval feeding and development, we conducted no-choice tests on 30 plant species, including *R. ellipticus* as a control (Tables 1 and 2). Five newly-hatched larvae of *E. tetragonana* or *E. ustulanan* were put onto leaves of the test plants in a Petri dish (11 cm diameter) with moist filter paper across the base. The dish was then sealed with Para-film. For most of the species, 15 larvae were tested (in three dishes). All the Petri dishes were checked and leaves were changed every 5 days, when the number of surviving larvae and their levels of feeding were recorded. If larvae survived, the experiments were continued until all the larvae pupated. Prior to the test with *E. ustulana*, seedlings of *Fragaria nilgerrensis* died. This species was then replaced with *D. indica*, which is in the same tribe with *F. nilgerrensis*.

In larval non-choice tests, differences in larval survival and pupation rates of both moth species were compared between control and treatment groups using chi-square goodness-of-fit tests performed on numbers of surviving insects in each stage.

2.2.3. Adult oviposition choice tests

Adult oviposition preference is the key to host selection of leaf-rollers. Both *E. tetragonana* and *E. ustulanan* larvae are leaf-rollers, living in the rolls until pupation. Individuals have limited opportunity to transfer from one plant to another so instead of larval-choice tests adult oviposition choice tests were conducted using plant species that supported larval development in the larval non-choice tests.

A total of 14 species were tested for *E. tetragonana*. Since most of the test species are bushes and hard to manipulate, tests were