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Assessment of a stenophagous weevil, *Osphilia tenuipes* (Coleoptera: Curculionidae), as a potential biological control agent for weedy *Bryophyllum* spp. (Crassulaceae) in Australia

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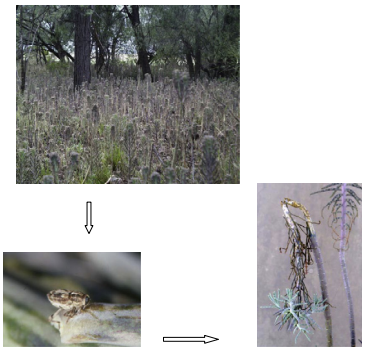
HIGHLIGHTS

- *Bryophyllum delagoense* is a target for biological control in Australia.
- The host range of the weevil *Osphilia tenuipes* confined to the Crassulaceae.
- It oviposits in *Kalanchoe blossfeldiana* in the presence of *B. delagoense*.
- Much of Australia is climatically suitable for *B. delagoense* and *O. tenuipes*.
- The insect will be assessed for release under Australia's *Biological Control Act*.

GRAPHICAL ABSTRACT



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ABSTRACT

Bryophyllum delagoense (Crassulaceae) is a serious weed in Australia and a target for biological control. Following preliminary testing in South Africa, the host range of a Madagascan conderine weevil, *Osphilia tenuipes*, was determined within a quarantine facility in Queensland, Australia. The primary test consisted of exposing potted plants, selected from an approved host test list, to attack by the weevil in a no-choice design. Tests of oviposition on cut stems and adult preference in a choice design were also undertaken. *Osphilia tenuipes* used all *Bryophyllum* spp. found in Australia as hosts and also several other exotic species of Crassulaceae, including *Kalanchoe blossfeldiana*, *Kalanchoe spathulata* and *Echeveria* sp. Native Australian species of Crassulaceae, all *Crassula* spp., were not attacked, nor were any plant species outside the family Crassulaceae. The preference test showed that the weevil fed on and oviposited in *Bryophyllum pinnatum*, *K. blossfeldiana* (two varieties) and *Kalanchoe sexangularis* in the presence of *B. delagoense*. The climate matching software CLIMEX was used to develop models that indicated that much of Australia, including southern states, would be climatically suitable for both *B. delagoense* and *O. tenuipes*, and that the predicted distribution of both organisms would move southward with climate change. Because the conflicts of interest attributed to the non-target attack are thought to be resolvable, the insect is to be assessed for release under the provisions of Australia's *Biological Control Act*.

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1. Introduction

The Madagascan plant genus *Bryophyllum* Salsb. (family Crassulaceae) is now represented in Australia by six species and a hybrid which have naturalized after their introduction as ornamentals (Hannan-Jones and Playford, 2002). *Bryophyllum delagoense* (Eckl. & Zeyh.) Schinz, known as mother-of-millions, and its hybrid *Bryophyllum* × *houghtonii* (D.B. Ward) P.I. Forst., known as hybrid mother-of-millions (Forster, 2006), have become increasingly-serious weeds in Queensland and northern New South Wales over the past 50 years, and are now declared weeds in both states. The remaining species, *Bryophyllum daigremontianum* (Raym.-Hamet & H. Perrier) A. Berger, *Bryophyllum beauverdii* (Raym.-Hamet) A. Berger, *Bryophyllum fedtschenkoi* (Raym.-Hamet & H. Perrier) Lauz.-March., *Bryophyllum pinnatum* (L. f.) Oken and *Bryophyllum proliferum* Bowie are regarded as both minor ornamentals and minor weeds in Australia. Another Madagascan genus, *Kalanchoe* Adans., is closely related to *Bryophyllum*, such that some authors (Baldwin, 1938; Boiteau and Allorge-Boiteau, 1995; Gehrig et al., 2001) consider all *Bryophyllum* spp. within a broadly circumscribed *Kalanchoe* (Hannan-Jones and Playford, 2002). Three species, *Kalanchoe longiflora* Schltr. ex J.M. Wood, *Kalanchoe lateritia* Engl. and *Kalanchoe spathulata* DC., but reported as *Kalanchoe crenata* (Andrews) Haw. (Kenneally, 1983), are also now naturalized in Australia. Several other *Kalanchoe* spp. are grown as ornamentals but have not naturalized. *Crassula* is the only genus of the Crassulaceae with species endemic to Australia where it is represented by eight native and many exotic species.

Mother-of-millions and hybrid mother-of-millions were considered to be sufficiently serious weeds to justify a project to find biological control agents. The project involved exploration for potential biological control agents in the country of origin, Madagascar (Witt and Rajaonarison, 2004) and also southern Africa (Witt et al., 2004).

The Madagascan conderine weevil *Osphilia tenuipes* (Fairmaire) (family Curculionidae) was first associated with *B. delagoense* in 1990 (J. Marohasy, unpublished data). Following surveys in Madagascar in 1999 where the insect was collected from only *B. delagoense* and *Bryophyllum pubescens* (Baker) Govaerts (Witt, 2004; Witt and Rajaonarison, 2004) the insect was brought to the South African Field Station situated at the ARC – Plant Protection Research Institute in Pretoria, where biology studies and preliminary host tests (Witt, 2004) were undertaken in quarantine facilities. These host tests, using 16 plant species (drawn from Crassulaceae, Lamiaceae and Portulacaceae) indicated that the host range of the insect did not extend beyond the Crassulaceae (Witt, 2004). It was therefore brought to Australia for additional host testing against an approved host test list. If considered adequately host-specific, permission would then be sought to release it in Australia as a biological control agent for mother-of-millions. Climatic suitability of a prospective agent for the areas where the plant is problematic in the introduced range is a major factor contributing to the agent's ultimate success and in relatively recent years climate-matching software has become available. This paper reports studies on the biology, host specificity and climatic suitability of *O. tenuipes* to support an application to release the insect in Australia. During the course of the described studies it became evident that the insect could not be released in Australia without declaration under the *Biological Control Act* and this is also discussed.

2. Materials and methods

2.1. The laboratory culture

A laboratory colony of *O. tenuipes* was established in the quarantine facility at the Alan Fletcher Research Station, Sherwood (a suburb of Brisbane), from material imported from the South African Field Station in May and July 2000. The South African colony was itself established from 37 adults that emerged from infested stems of *B. delagoense* collected in southern Madagascar in August 1999. The laboratory colony at the Alan Fletcher Research Station was maintained on potted plants of either *B. delagoense* or *B.* × *houghtonii* for the duration of the study.

2.2. Biology

2.2.1. Fecundity and longevity of adults

Freshly-emerged adults were observed closely and six mating pairs were captured and introduced into individual small plastic boxes, each containing a 8–10 cm length stem of *B. delagoense*. The stems were removed daily and replaced with fresh material. The number of feeding punctures on each removed stem was counted. The epidermal layer around the feeding marks was then carefully removed to expose the eggs inserted within the tissues of the plant. These eggs were then counted. This process was continued until all the adults died. The dead adults were then dissected under a binocular microscope to confirm their sex.

2.2.2. Larval stages

At fortnightly intervals, new cohorts of *O. tenuipes* were initiated so that at any one time there were several cohorts present and these provided a continual supply of insects for experiments. These were utilized to understand the insect's lifecycle. Two plants were sampled from individual cohorts to provide a range of ages (9, 12, 13, 20, 21, 28, 38, 41 and 47 days after first exposure of the plants to the adults) for immatures of the insect. The stems were dissected and the number of eggs, larvae, pupae and adult emergence holes were counted. Larvae were stored in 70% alcohol and at a later date, the widths of their head capsules were measured. Estimates of the duration of the immature stages were also made.

2.2.3. Cold tolerance

Several observations were undertaken to determine the cold tolerance of *O. tenuipes* and thereby improve the quality of climate matching modelling. The following treatments were applied:

(i) Twenty adults were placed in a freezer for 3 h at −15 °C. Mortality was assessed 4 h after removal from the freezer.
(ii) Twenty newly emerged adults were placed in each of four plastic boxes with sprigs of *B. delagoense*. Two of the boxes were placed in a temperature cabinet for two days of −3 °C for 8 h and 27 °C for 16 h, while two boxes were kept in the glass house (12 h each of 22 °C and 27 °C per day) as a control. Mortality of the weevils was assessed after the two days.
(iii) Two plants, each infested with immature larvae (30–35 days old) in their stems, were placed in a temperature cabinet for three days at −3 °C for 8 h and 27 °C for 16 h, while two similar plants were kept in the glass house (12 h each of 22 °C and 27 °C per day) as controls. On the fourth day the stems of all plants were dissected, live and dead larvae counted, and head capsule widths of all the larvae were measured.
(iv) Two plants, each infested with larvae (30–35 day old) were placed in the temperature cabinet for 7 days of 6 °C for 12 h and 16 °C for 12 h while controls were kept in the glass house (12 h each of 22 °C and 27 °C per day). After completion, the stems of all plants were dissected, live and dead larvae counted, and head capsule widths of all larvae were measured.
(v) Two plants infested with larvae (30–35 day old) were placed in the temperature cabinet for 7 days of 6 °C for 18 h and 12 °C for 6 h (simulating some of Melbourne's most severe