BIOLOGICAL CONTROL OF *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE)

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or any other examination in any other University.

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ABSTRACT

Introduced plants will lose interactions with natural enemies, mutualists and competitors from their native ranges, and possibly gain interactions with new species, under new abiotic conditions in their new environment. The use of biocontrol agents is based on the premise that introduced species are liberated from their natural enemies, although in some cases introduced species may not become invasive because they acquire novel natural enemies. In this study I consider the potential for the biocontrol of *Bryophyllum delagoense*, a Madagascan endemic, and hypothesize as to why this plant is invasive in Australia and not in South Africa.

Of the 33 species of insects collected on *B. delagoense* in Madagascar, three species, *Osphilia tenuipes*, *Eurytoma bryophylli*, and *Rhembastus* sp. showed potential as biocontrol agents in Australia. Surveys in southern Africa revealed that *B. delagoense* had acquired 14 species of insects, compared to only two in Australia, which supported the hypothesis that an introduced plant is less likely to become invasive in regions where there are many closely related species. A beetle, *Alcidodes sedi*, was found to be widespread and damaging in South Africa and also sufficiently host specific for release in Australia.

Studies were also undertaken to determine the biology of *B. delagoense* and characteristics of the recipient environments in Australia and South Africa. Analysis of soils in South Africa found that *B. delagoense* has the ability to grow on a range of soils and is tolerant of moderate shade. *Bryophyllum delagoense* exhibited phenotypic plasticity by re-allocating resources to stem production in light-limited; and to root production in nutrient- or water limited, environments. Competition trials indicated that *B. delagoense* is a weak competitor with overgrazing and the concomitant reduction in fires facilitating invasions. The absence of damaging natural enemies and characteristics of the Australian environment may make it more prone to *B. delagoense* invasions than in southern Africa.

The study indicates that no single trait or group of traits can completely explain the invasive ability of a plant species because the success of an invader often depends on complex interactions between the species and its recipient environment. This has widespread implications for predicting the invasibility of imported plants.

Keywords: *Alcidodes sedi*, biological control, *Bryophyllum delagoense*, Crassulaceae, *Eurytoma bryophylli*, fire, invasions, Madagascar, *Osphilia tenuipes*, *Rhembastus* sp., soil.

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CHAPTER 1

INTRODUCTION AND THESIS OUTLINE

Human activities such as trade, travel and transport, promote both the intentional and unintentional spread of species across their natural dispersal barriers. Out of those that are introduced, some species will establish, become naturalized, and then spread to invade communities and ecosystems to the detriment of biodiversity, food security, human health, water resources, provision of ecosystem services and economic development. Damage and control costs of invasive species is estimated to be US$1.4 trillion per annum, approximately 5% of Global Domestic Product in 2001 (Pimentel, 2001). Concern over the ecological and economic impacts of biological invasions has generated a lot of interest in the factors that contribute to invasion success (Crawley, 1987; Rejmánek and Richardson, 1996; Pyšek and Richardson, 2006). Numerous studies have considered the traits of successful invaders (Baker, 1965; Rejmánek and Richardson, 1996; Rejmánek, 1996; Kolar and Lodge, 2001; Catford *et al*., 2009) and the characteristics of recipient environments which make them susceptible to invasions (Rejmánek, 1989; Levine and D'Antonio, 1999; Mack *et al*., 2000; Pyšek and Richardson, 2006; Catford *et al*., 2009). According to the "Evolution of Increased Competitive Ability Hypothesis" (Blossey and Nötzold, 1995) the competitive ability of introduced plants can also increase over time and as such facilitate invasions. Although an introduced species may lose all of its natural enemies, competitors, and mutualists it will encounter novel species of potential enemies, mutualists, and competitors in its introduced range (Richardson *et al*., 2000, Levine *et al*., 2004). New damaging natural enemies, absence of mutualists, superior competitors and a resilient environment may prevent an introduced species from becoming invasive. It is critical to evaluate all of these aspects in determining if a species has the potential to become invasive. According to the "Enemy Release Hypothesis" (Keane and Crawley, 2002) the absence of natural enemies in the introduced range is often considered to be the main factor which allows introduced species to become invasive (Elton, 1958;

Colautti *et al*., 2004). However, the introduction of host-specific natural enemies into the new range of the invasive plants can reduce and in many cases negate their negative impacts on the environment. Biological control is increasingly being considered as a viable control strategy of alien weeds in many countries. In this dissertation, I evaluate the potential for the biological control of *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz (Crassulaceae) (mother-of-millions, finger plant) and which species traits, and what characteristics of the recipient environment contribute to the invasive status of this species in Australia.

Bryophyllum delagoense is an erect, unbranched herb with cylindrical succulent leaves endemic to Madagascar. It has been introduced as a garden ornamental to many countries worldwide, including Australia, where it has spread over most of Queensland in the past 30 years (Hannan-Jones & Playford 2002). The plant is considered allelopathic (Hannan-Jones & Playford 2002) and consumption of its flowers by cattle has been known to cause mortality (McKenzie & Armstrong 1986). The autecology of this plant is described in the following chapters and therefore not covered extensively in the introduction to avoid repetition.

This dissertation consists of 12 chapters, nine of which have been submitted for publication in various journals, of which six have been published to date. Because this thesis has been submitted in the form of publications, each chapter representing one manuscript, the information pertaining to sites and methodologies is often repeated in each chapter. The same applies to references where there is some duplication in each chapter. The references for Chapter 1, 2 and 12 and those cited in the justifications for the other chapters are only listed in the reference list at the end of this thesis. The justifications also provide information on the contributions of the various authors – it should be noted that I did all of the conceptualization and the bulk of the writing and analysis. The formatting for each chapter also differs depending to on which journal it has been submitted to, or published in.

Chapter 2 provides a statement of the research hypothesis, a rationale for the research and a review of the literature pertaining to invasive species in the family Crassulaceae, with particular reference to *B. delagoense*. I also review some of the literature on biological control and those papers which report on the various traits of invasive species and characteristics of the recipient environments which make them more prone to invasion. This chapter has not been submitted for publication.

In chapter 3, I report on the outcome of surveys for natural enemies associated with *B. delagoense* in Madagascar. Seventy-five *B. delagoense* localities in southern Madagascar were surveyed at least once during four survey trips over a two-year period. Closely related plant species were also surveyed in order to determine the field host range of some of the insects selected as potential biological control agents. The potential of three of the species of insects collected as biocontrol agents for *B. delagoense*, *Osphilia tenuipes* (Fairemaire) (Coleoptera: Curculionidae), *Eurytoma bryophylli* Neser (Hymenoptera: Eurytomidae) and *Rhembastus* sp. (Coleoptera: Chrysomelidae) are briefly discussed. Please note that *E. bryophylli* was only recently described (see Neser, 2008) – as a result this species is referred to as *Eurytoma* sp. in all chapters published prior to 2008.

Chapter 3 has been published in the journal “African Entomology”:

Witt, A.B.R. and Rajaonarison, J.H. (2004) Insects associated with *Bryophyllum delagoense* (Crassulaceae) in Madagascar and prospects for biological control of this weed. African Entomology 12(1): 1-7.

Chapter 4 follows in that it evaluates one of the three insect species, *O. tenuipes*, for its potential as a biological control agent. Biocontrol agents are selected based on a number of traits including their ability to build-up populations rapidly, their host range and impact on the host plant. The damage, host range and biology of potential agents are best determined under laboratory conditions supported by field observations (McFadyen, 1998). The host range of *O. tenuipes* was determined by undertaking no- and multiple-choice trials. It's potential as a biocontrol agent based on the results of laboratory trails and literature reviews are discussed.

Chapter 4 has been published in the journal "BioControl":

Witt A.B.R. (2004) Initial screening of the stem-boring weevil *Osphilia tenuipes*, a candidate agent for the biological control of *Bryophyllum delagoense* in Australia. BioControl 49:197-209.

In chapter 5 another species of insect, *E. bryophylli*, is evaluated for its potential as a biocontrol agent. An additional species was selected for further study because the more host specific and damaging agents that can be introduced to a country the higher the probability that the target weed will be controlled (Baars and Heystek, 2003; DeBach, 1964; Denoth *et al.*, 2002; Harris, 1981; Hoffmann and Moran, 1998; Schroeder and Goeden, 1986), a view supported in this thesis. In contrast, Myers (1985) concluded that in the majority of cases only one control organism does most of the damage while McEvoy and Coombs (1999) warned about the potential environmental risk in introducing a large number of agents. In this chapter I consider the natural distribution, life history, and host range of *E. bryophylli*. Data on the host range of other eurytomid species, including some crop pests, are also presented to support the results of the laboratory trials.

Chapter 5 has also been published in the journal "African Entomology":

Witt A.B.R., McConnachie A.J. and Docherty S. (2004) Distribution and aspects of the biology and host range of *Eurytoma* sp. (Hymenoptera: Eurytomidae), a candidate agent for the biological control of *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz (Crassulaceae) in Australia. African Entomology 12(2):201-207.

Rhembastus sp. is another species of insect that was selected for further studies and in chapter 6 I evaluate its potential as a biocontrol agent for *B. delagoense*. I selected another potential agent because it is considered beneficial to release a number of agents to control a specific weed provided that they all complement each other and do not compete for the same resource (Denoth *et al*., 2002). Releasing many agents also improves the odds that at least one of the released agents will establish and be effective (Denoth *et al*., 2002; Schroeder and Goeden, 1986). Chrysomelids have immense potential as biological control agents and many species have been used in the control of weeds throughout the world (Syrett *et al*., 1996; Julien and Griffiths, 1998). This chapter provides details on the field and laboratory host range of *Rhembastus* sp. I report on and discuss the results of no-choice adult feeding trials, and no-choice and multiple-choice adult feeding and larval development trials. I also describe the morphology of *Rhembastus* sp. life history stages in detail, and the host range of other species in the subfamily Eumolpinae.

Chapter 6 has been published in the journal "Biocontrol Science and Technology":

Witt A.B.R., McConnachie A.J., Palmer W.A. & Grobbelaar E. (2006) Distribution, biology and host range of *Rhembastus* sp. (Coleoptera: Chrysomelidae), a candidate for the biological control of *Bryophyllum delagoense* (Crassulaceae) in Australia. Biocontrol Science and Technology 16(8): 859-869.

In chapter 7 I describe the results of surveys of the natural enemies associated with *B. delagoense* in southern Africa. These surveys were undertaken because host range trials of the three species of insects collected in Madagascar, described in chapters 4, 5, and 6, revealed that they have the potential to also develop on *Kalanchoe* species. This is acceptable because there are only eight indigenous species in the family Crassulaceae, all belonging to the genus *Crassula* in

Australia (Tölken, 1981) with one possible exception. *Kalanchoe crenata* has been recorded from north-western Western Australia, but it is unclear whether this species is native or introduced to Australia (Kenneally 1983). In this thesis *K. crenata* is considered to be introduced to Australia. Insect species in southern Africa associated with native *Kalanchoe* and not *Crassula* species that have formed "new assocations" with *B. delagoense* may therefore have potential as biocontrol agents in Australia. Introduced plant species are more likely to acquire natural enemies from closely related plant species in the country of introduction (Hokkanen and Pimentel, 1989; Colautti *et al.*, 2004). I used data collected during the surveys to answer the question if biotic resistance in the form of interactions with new generalists or pre-adapted natural enemies, or rather a lack of suitable mutualists, may be preventing *B. delagoense* from becoming as invasive in its introduced range in southern Africa as it has in Australia. Phylogenetic relationships between native species in the family Crassulaceae and introduced *Bryophyllum* species in Australia, the United States, and southern Africa and the natural enemies associated with them are compared to confirm the enemy release hypothesis.

Chapter 7 has been submitted to the journal "Austral Ecology"**.**

Witt ABR, McConnachie AJ, Stals R (submitted) Is biotic resistance preventing *Bryophyllum delagoense* (Crassulaceae) from becoming invasive in southern Africa? Austral Ecology.

Chapter 8 follows by investigating the potential of one of the natural enemies, *Alcidodes sedi*, found on *B. delagoense* in southern Africa, as a potential biological control agent for this invasive plant in Australia. The search for agents was based on the hypothesis of "new associations" (Hokkanen and Pimentel, 1984). This hypothesis suggests that the probability of biocontrol success in using a biocontrol agent that originates from a congeneric host, and that is not naturally associated with the target in its native range, would be approximately

75% greater than with the so-called ‘old-association’ approach (Hokkanen and Pimentel, 1984). I report on the natural distribution, biology, and host specificity of *A. sedi*. In biology trials I consider rearing, development, feeding damage, dispersal ability, survival and longevity in detail and base host range on the results of field surveys and no-choice trials. I also investigate the phenomenon of “new associations” in more detail and the potential value of *A. sedi* as a potential biocontrol agent.

Chapter 8 has been published in the journal “Biological Control”:

Witt A.B.R., McConnachie A.J. & Stals R. (2004) *Alcidodes sedi* (Col.: Curculionidae), a natural enemy of *Bryophyllum delagoense* (Crassulaceae) in South Africa and a possible candidate agent for the biological control of this weed in Australia. Biological Control 31: 380–387.

In the previous chapters I consider biotic factors, mainly the influence of natural enemies, as determinants of invasion success or failure. In chapter 9 I consider habitat compatibility, in this case the influence of soil types and another biotic factor, competition, on the success or failure of *B. delagoense* as an invasive species. Abiotic characteristics of a habitat, including both climatic and edaphic conditions, have long been regarded as a fundamental determinant of the potential distribution of introduced plant populations (Mitchell *et al.*, 2006). Introduced species that do not have specific soil or mineral requirements should have the ability to establish and spread on a wide range of soil types within their introduced ranges (Huenneke *et al.*, 1990). I compared the particle-size distribution, pH and elemental composition of soil samples collected from seven *B. delagoense* sites, at five field localities in southern Africa. The growth of plants in these soil types were compared under laboratory conditions. Competition from resident species is another principal mechanism for biotic resistance to invasion (Catford *et al.*, 2009). To determine the competitive ability of *B. delagoense* I grew plantlets together with a grass species, *Eragrostis tef*, in two different soil types under two different watering regimes, measuring plant size at regular intervals. I then used the findings of this study to ascertain which factors may be responsible for the contrasting situation with regard to the invasiveness of *B. delagoense* in southern Africa and Australia.

Chapter 9 has been submitted to the journal “Biological Invasions”.

Witt ABR, Nongogo A, Byrne M (submitted) The influence of soil types and competition on the Madagascan endemic *Bryophyllum delagoense* (Crassulaceae) in South Africa, an invasive species in Australia. Biological Invasions

In chapter 10 I tested the effect of light intensity and soil type on *B. delagoense* growth rates in an attempt to explain the observed pattern where *B. delagoense* grows predominantly under trees in the Eastern Cape Province, South Africa. It also provided an opportunity to ascertain if *B. delagoense* supports optimal partitioning models by shifting resource allocation to leaf and stem production in light-limited environments, and to root production in nutrient-limited environments. The particle-size distribution, pH and elemental composition of soils growing in the shaded areas under trees and in the unshaded areas between trees were compared. Plantlets were grown in both soil types, under laboratory conditions, and various growth parameters recorded. Plantlets were also grown under different shade intensities to ascertain if *B. delagoense* can tolerate lower irradiance levels. The ability to grow under different light intensities could be considered an important attribute of invasive plant species, facilitating establishment in a wide range of habitats (Parendes and Jones, 2000; Lososová *et al*., 2007). Shade tolerance is especially important for short weed species (Lososová *et al*., 2007), such as *B. delagoense*, which are often overtopped by larger plants.

Chapter 10 has been submitted to the journal “Biological Invasions”.

Witt ABR, Nongogo A (submitted) Shade tolerance in the introduced plant *Bryophyllum delagoense* – fact or fantasy? Biological Invasions.

Habitat disturbance such as floods, fires and tree-falls may make ecosystems more prone to invasion. In chapter 11 I ask if *B. delagoense* is sensitive to fires and if a reduction in the frequency and intensity of fires may be contributing to the it’s invasive potential. Trials were undertaken to determine the sensitivity of *B. delagoense* to fires. I hypothesize that overgrazing and other factor’s may be contributing to a reduction in fires and as a result facilitating the continued spread of this weed. I also compare my results to those of other studies that looked at the impact of fire on other succulents, particularly invasive *Opuntia* species, several species of which have become invasive in Africa and Australia. I speculate as to why *B. delagoense* is more invasive in Australia than in southern Africa based on the results of this study.

Chapter 11 has been punblished in the journal “Biological Invasions”.

Witt ABR, Nongogo AX (2011) The impact of fire, and its potential role in limiting the distribution of *Bryophyllum delagoense* (Crassulaceae) in southern Africa. Biological Invasions 13:125-133.

Chapter 12 provides an overview of the project and how the findings have contributed to an improved knowledge of the natural enemies associated with various species in the family Crassulaceae. The study also provides invasion biologists with further insights into the biology and ecology of *B. delagoense* and which traits of the species and characteristics of the recipient environment facilitate its spread in Australia. It represents a complete case study of an invasive alien plant species, from foreign exploration for potential biocontrol agents and host range testing, through to plant physiology and ecology and characteristics of recipient environments, a study which has rarely ever been done for other weed control programmes. More importantly it highlights the risks of assuming that because an introduced plant species is not invasive in one country, even though it has been present there for more than 170 years, it will not become invasive if introduced elsewhere. The absence of natural enemies which can largely be attributed to the lack of closely related native species in Australia and characteritics of the recipient environment are key aspects contributing to its invasiveness in Australia – this may also be the case for other countries.

CHAPTER 2

LITERATURE REVIEW

Because only a small percentage of introduced species ever become invasive (Williamson, 1996) research has focused on trying to understand what makes some introduced species invasive and others not (Crawley, 1987; Richardson and Pyšek, 2006). Studies have examined the traits of successful invaders (Elton, 1958; Rejmánek, 1996; Rejmánek and Richardson, 1996) and the types of communities most susceptible to invasion (Levine and D'Antonio, 1999; Rejmánek, 1989). According to Catford *et al*., (2009) invasion is essentially a function of propagule pressure, the abiotic characteristics of the invaded ecosystem and the characteristics of the recipient community and invading species.

Research has not only focused on the theory of invasion biology but also on control methodologies, one of which, biological control, developed as a result of the enemy release hypothesis which attributes the success of invaders to the absence of natural enemies in the recipient environment (Colautti *et al*., 2004; Keane and Crawley, 2002; Joshi and Vrieling, 2005). According to DeBach (1964) biological control is the "actions of parasites, predators, and pathogens in maintaining another organism's density at a lower average than would occur in their absence." Classical biocontrol is the importation and release of exotic biocontrol agents, with the expectation that the agents will establish and further releases will not be necessary.

Biological control of weeds has been practiced for more than 90 years and introductions of biocontrol agents have been made against more than 50 weeds in more than 20 countries without a major mishap and with many beneficial results (Goeden, 1978; Julien, 1989; Julien and Griffiths 1998; Moran *et al*., 2005; Olckers, 1999; Schroeder, 1980). By 1990 the number of weed species targeted for biocontrol had increased to 54, 45, 28, 18, and 15 in the United States, Australia, South Africa, Canada, and New Zealand, respectively (Hoffmann, 1990). In South Africa the number of weed species targeted for biocontrol was 47 by 1999, with more than 85 species of biocontrol agents having been released (Olckers, 1999). Where host specificity testing has been done, according to universally accepted protocols, few unpredictable results of natural enemy introductions for biological control have occurred (Andres, 1981; Julien, 1989; Moran *et al*., 2005).

Before a biological control programme can be initiated it is critical to evaluate the probability of success. The decision on whether a weed is a suitable target for a biocontrol programme is based on the benefits to be achieved plus estimates of the probability of success (Sheppard *et al*., 2003; Wapshere *et al*., 1989). The more widespread and damaging the weed the greater the potential benefits in controlling it. On the other hand the probability of success largely depends on finding a host-specific and damaging agent, which establishes and inflicts significant damage on the target species in the country of introduction. According to Schroeder and Goeden (1986) biological control should be used in situations where conventional chemical, cultural and other traditional methods are difficult and not economic. The second criterion is evaluating the probability of success and this largely depends on the damage that each individual agent can do to the plant; the ecology of the biocontrol agent; and the ecology of the weed (Cullen, 1995).

Estimating the probably of biocontrol success is extremely difficult (McFadyen, 1998). Introduced agents can fail to establish or build up significantly high population numbers as a result of high parasitism levels (Hill and Hulley, 1995) and/or climate incompatability (Byrne *et al*., 2002; Byrne *et al*., 2004). Biocontrol of trees is believed to be particularly difficult despite there having been a number of successes (Dennill and Donnelly, 1991; Dennill *et al*., 1999; Hoffmann and Moran, 1999; Mack, 1996; Morris, 1999). It is also believed that biocontrol is unsuitable for weeds of annual crops or other frequently disturbed environments (Crawley, 1989; Duke, 1997; Reznik, 1996), yet some agricultural weeds have been successfully controlled (Chippendale, 1995; Hoffmann *et al*., 1998; Marsden *et al*., 1980; Morin *et al*., 1996). *Xanthium occidentale* L. (Asteraceae; Noogoora burr) was successfully brought under control in parts of Australia by the illegally or accidentally introduced leaf rust *Puccinia xanthi* Schw. (Pucciniaceae) (Chippendale, 1995). The introduced agent *Leptinotarsa texana* Schaeffer (Chrysomelidae) severely stunts the vegetative growth and fruiting capacity of the agricultural weed *Solanum elaeagnifolium* Cavanilles (Solonaceae; silverleaf nightshade) despite initial reservations of controlling a weed in arable lands (Hoffmann *et al*., 1998).

Biocontrol of species in the families Poaceae and Cyperaceae, and other herbaceous perennials with relatively low leaf nitrogen contents have also largely been unsuccessful (Crawley, 1989). This is compounded by the fact that the lack of secondary compounds or metabolites in grasses has created the perception that most natural enemies associated with them are generalists (Kuldau and Bacon, 2008).

Highly polymorphic species, such as *Lantana camara* (Verbenaceae; lantana), with numerous morphological and chemical phenotypes, which occur over large geographic areas, are also difficult to control (Crawley, 1989). Most of the insect agents released to date have restricted host ranges within this complex and in many cases are not able to establish throughout the range of *L. camara* (Thomas and Ellison, 2000). For example, in laboratory trials the agent *Longitarsus bethae* Savini & Escalona (Chrysomelidae) caused more damage and more readily colonized the *L. camara* varieties 150 Orange, 009 Light Pink and 113 Dark Pink while variety 018 Dark Pink was the least damaged, while 021 Total Pink and 029 White Pink were the least colonized (Simelane, 2006).

Burdon and Marshall (1981) concluded that inbreeding species were more likely to be controlled because there would be less morphological and chemical variation within the target species making all populations susceptible to attack by introduced biocontrol agents.

It is also generally accepted that the successful biocontrol of a weed in one country greatly increases the probability of success in another (Peschken and McClay, 1995) provided that the agent is suitably host specific and poses no threat to native species or crops. If the weed has close relatives in the proposed country of introduction the probability of it being released are considerably reduced unless the agent is monophagous, which is comparatively rare (Olckers *et al*., 1995).

The first step in any biological control programme should be the correct identification of the target weed species and related species (Gaskin and Schaal, 2002; Goeden, 1978; Schroeder, 1983). It is critical to determine the correct biotype, failure to do so may result in the agent failing to establish due to plant-natural enemy incompatability (Tracy and Robbins, 2009; Volchansky *et al*., 1999; Zachariades *et al*., 1999). The importance of this was demonstrated in the case of *Chondrilla juncea* L. (Asteraceae; skeletonweed) in Australia where the rust *Puccinia chondrillana* (Pucciniaceae) only infected the narrow-leaf form of this weed and failed to establish on the other two forms (Hasan, 1972). Host-agent incompatibility has also hampered the effective control of *Euphorbia esula* L. (Euphorbiaceae; leafy spurge) in North America (Gassmann and Schroeder, 1995) and it is suspected that that the culturing of five agents introduced into quarantine laboratories in South Africa for the control of *Chromolaena odorata* (L.) King & Robinson (Asteraceae; triffid weed) failed due to host-agent incompatibility or incorrect breeding techniques (Zachariades *et al*., 1999).

After the taxonomy of the target weed has been confirmed it is essential to determine the current distribution of the weed and its potential range in the country of introduction (Harris and Cranston, 1979), any natural enemies associated with the target weed in the recipient environment (Andres, 1981), determine and address any potential conflicts of interest (Andres, 1981; Sheppard *et al.*, 2003), and estimate the economic losses associated with the weed (Harris and Cranston, 1979; Sheppard *et al.*, 2003). It is critical to determine which native natural enemies are associated with the invasive plant and which natural enemies, if any, may have been accidentally introduced with the weed (Goeden, 1971; Maw, 1976). This occurred with the biocontrol programme for *Sesbania punicea* (Cav.) Benth. (Papilionaceae; red sesbania) (Hoffmann and Moran, 1991). Four potential agents, including the weevil *Trichapion lativentre* (Bêguin-Billecocq) (Curculionidae) were introduced into quarantine in South Africa for the control of *S. punicea* (Hoffmann and Moran, 1991). A participant at a meeting subsequent to their introduction into quarantine laboratories, but before host range trials had commenced, reported that he had seen *T. lativentre* in the field in South Africa (J.H. Hoffmann, pers. comm.). As a result the *T. lativentre* quarantine culture was destroyed leading to considerable savings in potential research costs. This has not always been the case with some agents only being discovered in the field once host range testing has already been completed and the agent has been mass reared and released, a considerable waste of resources. For example, the broom stem and twig miner (*Leucoptera spartifoliella* Hűbner), a natural enemy of Scotch broom (*Cytisus scoparius* Link), was already present in the United States together with a major parasitic wasp that keeps the twig miner generally uncommon in Europe; both were introduced by accident (Frick, 1964). This highlights the importance of undertaking surveys for natural enemies associated with an invasive plant species in its introduced range prior to surveys in the native range.

It is suggested that surveys for potential biocontrol agents be undertaken in the centre of diversification of the invasive plant. This is based on studies which have demonstrated that the highest numbers of herbivorous natural enemies are found in the centre of diversification of a weed genus or subgenus (Goeden, 1971; Goeden and Ricker, 1978; Harris and Piper, 1970; Wapshere, 1974; 1981). Although this is advocated, it is not always possible to determine the centre of diversification of plant species (Holm *et al*., 1977) and habitat destruction has often drastically altered the distribution and abundance of particular plant species (Annecke and Moran, 1978; Holm *et al*., 1977). Hybridization between introduced and/or native species in the country of introduction can also inhibit the ability to locate suitable biocontrol agents in the country of origin (Gaskin and Schaal, 2002). A particular biocontrol agent may have to be sourced from various populations throughout its country of origin to improve the possibility of establishment on a genetically variable target species (Gaskin and Schaal, 2002; Tracy and Robbins, 2009). Attempts should also be made to undertake surveys in areas that are climatically similar to where the plant is invasive because the distribution of insects, mites and pathogens is largely determined by climatic variables (Dhileepan *et al*., 2006; Julien *et al*., 1995; Robertson *et al*., 2008). However, the centre of diversification may not be climatically analogous to the region where agents are intended for release (Schroeder and Goeden, 1986). Some areas may also not be accessible for political reasons (Schroeder and Goeden, 1986), poor infrastructure, and issues regarding biopiracy mean that access to some countries to collect agents is often denied. For example, South African biocontrol researchers were denied access to potential biocontrol agents in Brazil for many years.

The selection of biocontrol agents is critical because of the time and costs involved in overseas surveys as well as the host specificity screening of selected agents in quarantine laboratories (Schroeder and Goeden, 1986). In 1973, in Canada, it was estimated to cost at least one scientist-year ($40,000) to obtain and demonstrate that an agent was safe to release and several man-years of subsequent study if the agent established (Harris, 1973). According to Harris and McEvoy (1995) the cost of screening an agent in Canada in 1992 was about 2 scientist-years ($400 000). Studies in South Africa revealed the estimated cost of research into the biological control of six invasive plant species (in 2000 rand values) was R41.1 million (van Wilgen *et al*., 2004). Of this, 42% (R17.3 million) was spent on the biocontrol of *L. camara*; 43% on *Opuntia aurantiaca* Lindl. (Cactaceae;

jointed cactus) (R8.6 million) and *Hakea sericea* Schrader (Proteaceae; silky hakea) (R9.2 million); and only 15% on *S. punicea*, *Acacia longifolia* (Andr.) Willd. (Fabaceae; long-leaved wattle), and *A. pycnantha* Benth. (Fabaceae; golden wattle) (van Wilgen *et al*., 2004). The costs associated with a biological control programme are therefore largely dependent on the target species but also on the selection of specific and damaging biocontrol agents. Nevertheless costs are high so there is economic and in many cases also political pressure to choose the most appropriate agents during initial surveys. Because of potential environmental risks there is also pressure to release fewer agents (McEvoy and Coombs, 1999).

Wapshere (1974) recommended that the most effective agents would be those that caused the most damage in the natural distribution making allowances for minor ecological differences and for the role of parasitoids, predators and diseases. In reality, determining the impact of potential agents in their natural range is very difficult if not impossible (Schroeder and Goeden, 1986). The impact of potential agents and their natural enemies can be influenced by human activities, such as pesticide use, which make a valid estimation of the impact of herbivorous insects or mites often impossible (Schroeder and Goeden, 1986). Harris (1973) suggested a scoring system in order to assist with the selection of potentially the most effective biocontrol agent before host specificity studies commenced. The scoring system was based on the host specificity of the agent, the direct and indirect damage inflicted, phenology of attack, number of generations, number of progeny per generation, extrinsic mortality factors, feeding behaviour, compatibility with other agents, distribution, evidence of effectiveness elsewhere and size of the agent (larger agents were considered to be more effective). Goeden (1983) reviewed the scoring system proposed by Harris (1973) by dividing it into three major phases namely: initial assessment of destructiveness in native range which included damage in the field, phenology, number of generations, and distribution; suitability as a biological control agent which required the researcher to consider the host range and ease of culturing the proposed agent; and potential effectiveness in area of introduction which included ecoclimatic matching and colonization history, and by so doing improved its use as a predictive model.

Wapshere (1985) proposed the “ecoclimatic method” which considered the distribution and impact of agents on the host plant in their native range. According to Crawley (1986, 1987, 1988) a high intrinsic rate of increase is an important ecological characteristic of potential biocontrol agents and this tends to be associated with small body size (Southwood, 1981). A review by Crawley (1989) enabled decisions to be made on past successes and failures which confirmed that smaller insects were more likely to become established following introduction as biocontrol agents and that beetles, particularly weevils and chrysomelids, were more successful as agents than any other taxa.

The factors associated with high establishment probability are high rate of increase, long-lived adults, high voltinism, and low per-capita feeding rates associated with small individual size to correlate broadly with degree of success (Crawley, 1989). This has been disputed by Cullen (1995) who claimed that some slower-increasing species have also been effective. According to Cullen (1995) the success of an agent depends on three major factors: i) the damage that an agent (individual or population unit) can inflict on a plant; ii) the ecology of the agent in determining its density and therefore the total damage produced; and iii) the ecology of the weed in determining if that damage is significant in reducing its population. Similarities between the recipient and native environments are also important in terms of temperature and moisture (Byrne *et al*., 2002; Byrne *et al*., 2004), characteristics of the recipient environment (e.g., cultivation, loss of refuges, etc.) and predation/parasitism from native insects (Cullen, 1995; Hill and Hulley, 1995). This view was supported by Blossey (1995a) who stated that biocontrol success does not depend on the biology of the insect as much as its interaction with environmental factors such as climate and parasites or predators. Cullen (1995) cautioned against the use of generalizations in selecting agents because important exceptions may be neglected in the process. According to Thomas and Reid (2007) it is not possible to draw any major conclusions because unfortunately biocontrol programmes are generally poorly evaluated – we need a better ecological understanding of the impacts of the agents and the target species themselves. In the final analysis, often as a result of financial constraints, it is often the agents that are most readily available and easily studied in the weed's country of origin that are selected as potential biocontrol agents (Harris and Zwölfer, 1968; Zwölfer and Harris, 1971).

Hokkanen and Pimentel (1984) suggested that natural enemies associated with the invasive plant in the country of introduction would be more effective as biocontrol agents than those sourced from the country of origin. It was claimed that the evolved balance in the old association among parasites and their host may prevent them from being effective biological control agents (Pimentel, 1963). A review of the data tentatively suggested that new associations between enemy and pest were 2.2 times more successful for control of weedy plants (Hokkanen and Pimentel, 1984). According to Hokkanen and Pimentel (1984) new associations should be utilized as the primary means of selecting natural enemies for biocontrol, because they offered greater chances of success than those agents that had a long co-evolutionary with the target species. This approach was rejected by Goeden and Kok (1986) who found that examples of biological control of weeds cited in their paper (Hokkanen and Pimental, 1984) were biased towards cactaceous insects and others were inaccurate. They stated that the preferred method should continue to be the current practice, that natural enemies of an alien weed are best sought in the country of origin. However, it was found that herbivore-plant associations in agricultural situations, which are analogous to agent-weed associations, supported the hypothesis of new associations (Dennill and Moran, 1989). Of the 40 most important agricultural pests in South Africa, 58% are in new associations (Dennill and Moran, 1989). The authors concluded that new associations between herbivore species and host plants have strong potential in weed biocontrol, which is in agreement with Ehler (1995). However, the success of new associations is not necessarily because of the absence of coeveolved “homeostasis”, as suggested by Hokkanen and Pimentel (1984), but other factors such as the presence of parasitoids, predators, and/or competitors that may be limiting the impact of a natural enemy, on taxonomically-related target pests (Ehler, 1995). The impact of crop pests, including those in new associations, may be enhanced by the impact of pesticide use on native predators and/or predators, a factor which was not considered by Dennill and Moran (1989). Ehler (1995) subsequently suggested that the conventional dichotomy of new vs. old was insufficient to characterize the nature of pest-enemy associations and proposed four types of associations which he called “new, recent, quasi-old, and old”. Ehler (1995) concluded that the selection of an agent or introduction strategy required a blend of “induction, deduction, experience, intuition, and inspiration.”

The host specificity of those agents selected for further study is a critical phase in any classical biological control of weeds project. According to Schroeder and Goeden (1986) the initial screening and most host specificity studies are usually undertaken in the native range of the target weed. There are good reasons for doing this because expensive quarantine facilities are not required for mass rearing and host range testing, plants closely related to the target species can be sourced from the field, and laboratory trials can be complemented with field trials (Schroeder and Goeden, 1986). Although it may be cheaper to undertake studies in the country of origin it does mean that related native plant species in the country of introduction cannot be tested in many situations because their importation may pose a risk to biodiversity should they escape, establish and spread. Researchers from institutions in countries such as Australia and New Zealand are now legally obligated to at least undertake preliminary host range trials in the agent’s country of origin, prior to introduction, to reduce the potential risks of an introduced agent establishing on non-target species should it escape from quarantine laboratories. This is why preliminary host range trials were undertaken in South Africa on all potential agents collected on *B. delagoense* in Madagascar and South Africa prior to exportation to Australia for futher testing.

Once the locality for testing has been established host specificity trials can commence which should follow the centrifugal phylogenetic method which entails the testing of the taxonomically most closely related species to the target plant first and progressively incorporating more distantly related plants until the host range of the natural enemy has been adequately described (Wapshere, 1974). Plants with morphological or biochemical characteristics similar to those of the target weed should also be tested (Harris and Zwölfer, 1968). With advances in molecular biology it has been proposed that information on phylogenetic relationships and insect host-choice evolution and behavior also be utilized in the selection of test species (Briese, 2005). At one stage it was also suggested that the list of test plants for every biocontrol programme initiated in North America should include all endangered or important native plants present in North America irrespective of their relationship to the target species (Andres, 1981). This "request" clearly had no scientific merit and for many plant species was not practical. The selection of test plants should be based on sound ecological and economic principles, "not simply on unrealistic demand for absolute safety of introduced biological control agents" (Schroeder and Goeden, 1986).

Oviposition, feeding, and nymphal and larval development tests involve single plants (no-choice), paired-choice (two species, one of which is the target species) or multiple choice tests using several species of plants. Feeding tests should focus on critical plant species and be complemented by starvation tests if necessary (Schroeder and Goeden, 1986). Harris and McEvoy (1995) felt that there were major deficiencies in using no-choice feeding tests alone to determine the safety of an agent and suggested that the lack of predictability of feeding tests arises because insect host selection is the result of a hierarchial sequence of opportunities and constraints. For example, the weevil species *Ceutorhynchus litura* (Fabricius) and *Trichosirocalus horridus* (Panzer) (Curculionidae), introduced for the control of thistles in North America, have very different field host ranges but almost identical laboratory host ranges probably because the adults differ in host selection while larvae from both species have the ability to develop on a wider and shared range of species (Harris and McEvoy, 1995). According to Harris and McEvoy (1995) host selection constraints should be divided into six groups: (i) phylogenetic constraints that are determined by evolutionary history; (ii) climatic constraints; (iii) habitat needs; (iv) oviposition needs; (v) host acceptability for feeding; and (vi) host suitability for development. All of these six steps should be addressed in a host selection process because basing decisions on laboratory host range trials alone could result in the rejection of perfectly suitable and effective biocontrol agents (Harris and McEvoy, 1995). The advantages and disadvantages of the various kinds of screening tests have also been discussed extensively elsewhere (Harris and Zwölfer, 1968; Zwölfer and Harris, 1971).

The major problem encountered, as raised by Harris and McEvoy (1995) is the interpretation of results in which feeding on non-target plants occurs in tests but not in the field (McFadyen, 1998). Such feeding may be an artifact of confinement, whereby restriction in a cage prevents normal host-finding behaviour (Balcunias *et al*., 1996; Harris and McEvoy, 1995). This is termed the physiological or behavioural host range because the tests are conducted under artificial conditions under optimal conditions that favour the biocontrol agent (Louda *et al*., 2003). The use of larger cages such as walk-in cages where the agent can be exposed to a range of plant species may result in more natural behaviour and more genuine results (Cullen, 1990). Open-field testing in the country of origin allows the insect to behave totally naturally (Briese *et al*., 1995; Clement and Cristofaro, 1995) and should provide the most realistic results in terms of host range, often termed the natural or ecological host range because the agent has to contend with physical and environmental limitations (Louda *et al*., 2003). However, agent populations in the country of origin are generally much lower than they would potentially be in the country of introduction due to the action of parasitoids, predators and competitors which means that predictions on agent behaviour and impact in the country of introduction are not always possible (Gassman and Schroeder, 1995). However, the use of mathematical models which combine host range data with genetic relationship data from known plant phylogenies may allow for improved predictions of the ecological host range of potential agents (Berner *et al*., 2009).

The interpretation of host range trials depends largely on the quality of knowledge collated during foreign surveys, as well as an understanding of the biology and ecology of the target weed (Louda *et al*, 2003; Schroeder and Goeden, 1986). Once host range testing has been completed Pest Risk Assessments (FAO, 2004; EPPO, 2006) are compiled and sent to the relevant authorities for approval (Sheppard *et al*., 2003). If they deem the agents to be safe for release they are mass reared and released into the recipient environment where they will hopefully establish and control the invasive plant species.

Common to all theories of invasion biology is the understanding that successful invasion requires sufficient propagule pressure (Catford *et al*., 2009) which is in effect the number of individuals introduced at any one time multiplied by the temporal frequency of these introductions (Eppstein and Molofsky, 2007). Propagule pressure is linked to residence time which may explain why plants that are repeatedly introduced over a wide area over a long period of time are more likely to establish and become invasive (Crawley *et al*., 1996; Rejmánek *et al*., 2005). This period between the initial introduction and when the plant becomes invasive is known as the lag phase (Binggeli, 2001; Kowarik, 1995). Kowarik (1995) reported that for 184 invasive species the average duration of time–lags was 131 and 170 years for shrubs and trees, respectively. The duration of time-lags is highly variable (Binggeli, 2001). According to Hobbs and Humphries (1995) time-lags are as a result of genotypic adaptations; cyclical disturbance or a combination of environmental conditions (abiotic factors); or species with exponential growth which is only noticed when the population reaches a critical size (biotic factors). Biotic factors could also include the introduction of pollinators or dispersers (Binggeli, 2001). In many cases there is no discernable ecological reason for a time-lag and this pertains especially to species which are linked with human activities, where the lag phase is clearly related to human transportation of a particular species (Binggeli, 2001). *Agrostema githago* L. (Caryophyllaceae; common corncockle) was formerly one of the most common weeds in croplands in many countries as a result of repeated introductions of seeds in contaminated cereal seed but with more effective cleaning of grains nowadays it is almost extinct in many areas where it was previously recorded as a weed (Rejmánek, 1999). However, an introduced plant will never establish if the environmental characteristics of a site are not suitable for invasion to occur.

Abiotic or environmental characteristics of a habitat have long been regarded as a fundamental determinant of the potential distribution of introduced plant populations (Mitchell *et al*., 2006). Introduced species that do not have specific soil or mineral requirements should have the ability to establish and spread on a wide range of soil types within their introduced ranges (Huenneke *et al*., 1990). Abundant weeds tend to be habitat generalists, occurring in several habitats (Lososová *et al*., 2007). Episodic disturbance events can also increase resource availability and make recipient environments more prone to invasion (Elton 1958; Rejmánek and Richardson, 1996). The overwhelming majority of invasions take place in human- and/or naturally-disturbed habitats (Hobbs and Huenneke, 1992; Mack, 1989; Rejmánek, 1989; Schiffman, 1997; Whitemore, 1991). These disturbance events may be facilitated by fire, floods, agricultural practices, livestock grazing, drainage of wetlands or alteration of salinity, and nutrient levels in water bodies (Mack *et al*., 2000). Disturbance events reduce the cover of adult plants increasing space for colonization, and reduce competition between native and introduced species (Crawley *et al*., 1999).

Many exotic plant species become more abundant and dominant as fire frequencies increase (Hamilton, 1997; Huenneke, 1989) but in some cases the absence of fires increases the prevalence of exotic species, especially succulents (D'Antonia, 2000). Disturbance events such as overgrazing may therefore facilitate exotic plant invasions and at the same time reduce the amount of flammable material to carry a fire, facilitating the invasion of ecosystems by fire-sensitive invasive plant species. However, even if the abiotic characteristics of the recipient environment are suitable for invasion, most introduced species still need to overcome biotic resistance, which is considered to be the reduction in invasion success caused by the resident community (Levine *et al*., 2004).

Introduced species can lose and gain biotic interactions in their recipient environments. Communities may resist invasion through a diversity of biotic processes, including predation, competition, herbivory or disease (Levine *et al*., 2004). Biotic interactions such as enemy release (Keane and Crawley, 2002), evolution of improved competitive ability (Blossey and Nötzold, 1995), allelopathy (Callaway and Ridenour, 2004) and, symbiosis (Richardson and Pyšek, 2006) can facilitate invasion while "biotic containment" (Levine *et al*., 2004) and interspecific competition (Burke and Grime, 1996) can inhibit invasion success. Some introduced plants may never become invasive in their introduced range because of the absence of suitable pollinators or mycorrhizal symbionts (Pringle *et al*., 2009). Many introduced organisms arrive in new localities without their native associates, including their competitors and natural enemies (Elton, 1958; Strong *et al*., 1984). As a result an invader can persist and proliferate not because it possesses a suite of extraordinary traits but because it has arrived in a new environment without its associated natural enemies and competitors (Mack *et al*., 2000). However, as an invasive species expands its range it is more likely to come into contact with a wider range of closely related native plant species, hosts to a multitude of phytophagous insects, mites and diseases, some of which may be pre-adapted to feed and develop on the introduced plant species (Strong *et al*., 1984).

It has also been hypothesized that the success of species belonging to non-native genera and families may be due to the limited number of resident herbivores and pathogens able to switch to species phylogenetically distant from their native hosts (Rejmánek, 1999). This was already reported on by de Candolle in 1855 who noted that naturalized species within a given region were distantly related to any native species. This was supported by Rejmánek (1996) who found that European species in the families Asteraceae, Fabaceae, and Poaceae that were naturalized in California were statistically more likely to belong to alien genera than expected from a random pool of European species. This led to a rigorous study of 20 plant families in Hawaii where it was found that introduced species less related to native species were not necessarily more invasive (Daehler, 2001). Daehler (2001) concluded that introduced species with close relatives, often in the same genus, have better chances of naturalizing because they share with their congeners some level of preadaptation to the conditions of the invaded region. The contrasting results may be due to differences between families and regions or because island and mainland communities are possibly regulated by different factors (Daehler, 2001). This has led to increased research of this particular hypothesis (Richardson and Pyšek, 2006).

The absence of natural enemies in the introduced range led to a theory by Blossey and Nötzold (1995) that invasiveness of non-native plants is as a result of shifts in biomass allocation from defense, such as the production of secondary metabolites, to improved competitive abilities. This may partly explain why alien plants are, on average, taller than natives, at least in some habitats (Pyšek *et al*., 1995; Crawley *et al*., 1996). Daehler and Strong (1997) reported reduced herbivore resistance to the delphacid *Prokelesia emarginata* (Van Duzee) (Delphacidae) in the introduced grass *Spartina alternifolia* Loisel. (Poaceae; smooth cordgrass). The enemy release hypothesis argues that many introduced species become invasive as a result of the absence or reduction of natural enemies in the introduced environment while the biotic resistance hypothesis argues that interactions with native species, limits the impacts of invasive species. A study by Mitchell and Power (2003) of viruses and pathogens on 473 naturalized plant species found on average 84% fewer fungi and 24% fewer virus species on each plant species in its naturalized range than in its native range. However, without suitable traits, invading species will not be able to benefit from favourable environmental conditions and the absence of natural enemies.

Many studies have been undertaken to determine what characters or combinations of characters are responsible for species invasiveness (Andersen 1995; Baruch and Gomez, 1996; Mazer, 1989; Pyšek *et al.*, 1995; Williamson and Fitter, 1996). Families with a disproportionately high representation of invasive species are Amaranthaceae, Brassicaceae, Convolvulaceae, Malvaceae, Poaceae, Papaveraceae, Polygonaceae, and Fabaceae (Daehler, 1998; Pyšek, 1998; Weber, 1997). Some of the worst woody plant invaders are to be found in the families Myrtaceae, Rosaceae, Salicaceae, and Tamaricaceae (Williams *et al.*, 2001). The first detailed study on the characteristics of an invasive species were undertaken by Rejmánek and Richardson (1996) based on the data available for 24 well known and frequently cultivated pine species, many of which are known to be invasive. Twenty-eight of the 36 gymnosperms known to be invasive worldwide belong to the family Pinaceae and 21 of these belong to the genus *Pinus* (Richardson and Rejmánek, 2004). The most important characters which differentiated between the non-invasive and invasive *Pinus* species were mean seed mass, minimum juvenile period, and mean interval between large seed crops. Small seed mass is usually associated with high seed production, improved dispersal, high initial germinability, and shorter chilling period needs to overcome dormancy while short juvenile period may be related to fast growth (Rejmánek and Richardson, 1996). Analysis of 40 invasive woody plant species also revealed that the seeds of the majority were dispersed by birds, bats or primates (Rejmánek and Richardson, 1996). Grasses are the second most important group of plant invaders (Baker, 1978; D’Antonio and Parsons, 1970; Huenneke and Mooney, 1989; Vitousek, 1992) due to low construction costs associated with high leaf area ratio and high specific leaf area (Baruch and Gomez, 1996).

Other factors such as self-pollination, breakdown of pollinator specificity and vegetative reproduction can also be important in the initial stages of invasion (Baker, 1967; Parrish and Bazzaz 1978). Lososová *et al*. (2007) found that the majority of weeds in central Europe had an extended flowering period which enabled them to increase their chances of cross-pollination and seed output. Vegetative reproduction is essential for establishment and short-distance dispersal of many species in terrestrial environments (Andersen, 1995; Auld *et al*., 1983; Pyšek 1997; Thompson *et al*., 1995). Vegetative reproduction is also important for long-distance dispersal in aquatic habitats and floodplains (Ashton and Mitchell, 1989; Bramley *et al*., 1995; Spencer and Rejmánek, 1989). The invasiveness of *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae; water hyacinth) can largely be attributed to its free-floating life form and asexual reproduction by stolons (Barrett, 1992). Rapid root growth (Hulbert, 1955), shade tolerance (Jones and McLeod, 1990), resistance to browsing (Ledgard, 1988), and production of allelochemicals (Lawrence *et al*., 1991) all contribute to invasiveness. According to Lososová *et al*. (2007) shade tolerance is very important for short weed species that are overtopped by larger crop plants. Successful arable weeds seem to occupy nutrient-rich but more shaded habitats (Tilman, 1988) which was confirmed by Lososová *et al*. (2007) who found Ellenberg light values for abundant weed species of mostly 6 and 7, while those for less abundant species of mostly 7 and 8 (Tilman, 1988). Although an introduced plant species can possess many traits which may facilitate its success as an invader, the plant must have sufficiently high levels of physiological tolerance and plasticity, or it must undergo genetic differentiation to achieve the required levels of fitness (Richardson and Pyšek, 2006).

Successful plant invaders generally occur over a wide region in their country of origin (Rejmánek, 1995) because they have the ability to tolerate a range of environmental conditions. These very same biological traits that enable some species to spread across their native geographic ranges over different climatic zones also make them able to invade new continents (Roy *et al*., 1991). The ability of an organism to maintain constant fitness over a range of environments is called "fitness homeostasis" (Hoffmann and Parsons, 1991) and can largely be attributed to phenotypic plasticity. A plant species which is grown under different environmental conditions and exhibits differences in morphological or physiological traits such as leaf characteristics (Ryser and Eek, 2000), photosynthesis (Thompson *et al.*, 1992), and overall patterns of biomass allocation (Chapin, 1991; Grime *et al*., 1986) is considered to be plastic, a trait that has long been considered as contributing to the success of many weeds (Baker, 1974; Roy, 1990). Plants which exhibit phenotypic plasticity can optimize growth by allocating resources from stems and leaves to root production in nutrient-poor soils and from roots to leaf and/or stem production in light-limited environments without any discernable impacts on fitness. This ability to maintain constant fitness under light-limited and nutrient-poor environments means that a wider range of habitats can be invaded (Parendes and Jones, 2000; Lososová *et al*., 2007). While there are presumably costs associated with being plastic, this ability may be another way invasive species can gain an advantage over indigenous species (Williams *et al*., 1995).

In the final analysis it is clear that the theory of invasion biology is developing rapidly while that of biological control has not developed beyond the ecological foundations laid down many years ago. As we gain a better ability to predict which plant species will invade and which receipient environments are receptive to invasions we still have an inability to predict which agents will establish, and of those that do, which will successfully control the target species. There are probably a multitude of reasons as to why this is the case but clearly biocontrol requires a paradigm shift.

In the following chapters I attempt to weave the concepts of plant traits and characteristics of recipient environments with that of biological control, using *B. delagoense* as a case study, in the hope that it may lead to a better understanding of the theory of invasion biology. I evaluate the potential for the biological control of *Bryophyllum delagoense* and determine which factors may be contributing to the invasive potential of this plant in Australia. Thanks to a host of factors, including the presence of a number of natural enemies, *B. delagoense* is not invasive in its native range in southern Madagascar or in southern Africa, where it has been introduced. In the following chapter I report on the number of phyophagous insects associated with *B. delagoense* in Madagascar and evaluate their potential as biocontrol agents.

CHAPTER 3

INSECTS ASSOCIATED WITH *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE) IN MADAGASCAR AND PROSPECTS FOR BIOLOGICAL CONTROL OF THIS WEED

Justification

Many introduced plant species become invasive because the natural enemies associated with the species in its country of origin are absent in the country of introduction (Rejmánek *et al*., 2005). It is possible to reduce the competitive advantage of invasive plant species such as *B. delagoense*, in their introduced range, by introducing host specific natural enemies or biological control agents (McFadyen, 1998). One of the first steps in such a programme, after the identification of the target weed and infraspecific taxa has been confirmed (Goeden, 1978), is to undertake surveys of the arthropods and pathogens associated with a plant species in its native range, nearest the centre(s) of diversification of the weed genus or subgenus (Gaskin and Schaal, 2002; Schroeder and Goeden, 1986; Tracy and Robbins, 2009). Surveys in a plant's native range also provide an opportunity to gain better insights into the biology of the target weed, information which may contribute to a better understanding of the processes that enable exotics to dominate recipient communities (Hierro *et al*., 2005). Once a complete list of all arthropods and pathogens has been compiled, the most promising agents, usually those that have multiple generations per year, are widespread, most damaging in the field, and considered to have a narrow host range, are selected for further study (Goeden, 1983; Harris, 1973).

This chapter provides novel information on the distribution, and natural enemies, of *B. delagoense* in Madagascar. It describes the research that was undertaken prior to and during surveys in Madagascar. A literature review provided little information with regard to *B. delagoense* localities in southern Madagascar (Boiteau and Allorge-Boiteau, 1995). There was also very little data available on the insects, mites and/or pathogens associated with *B. delagoense*, any of its congeners or other species in the family Crassulaceae, other than some insect species associated with ornamental crassulaceous species (Dickson, 1947; Griffiths, 1976; Kroon, 1976, 1999; Marrone and Zepp, 1979; Pringle *et al*., 1994; Schmid, 2000). Based on the architecture of *B. delagoense* and the number of phytophagous insects associated with other succulents, such as small, narrow-jointed cactus species (Moran, 1980), we assumed that we would only find approximately 10 species of insects that would be able to feed and develop on the target species. Intensive surveys in southern Madagascar revealed that our assumption was correct with the discovery of 10 species of insects. Three of these insect species were selected for further study because they were widespread, with *Osphilia tenuipes*, *Rhembastus* sp., and *E. bryophylli* present and/or damage visible at 52%, 55%, and 23% of the 75 sites surveyed, respectively. They were also abundant at many of the localities, had multiple generations per year and were only found on closely related plant species in the field.

Surveys also gave the authors insights into the phenology of *B. delagoense* and the insects associated with it, as well as information on the habitats in which *B. delagoense* occurs, and other plant species that grow in association with it. This also provided valuable information which contributed to the formulation of various hypotheses regarding the role of soil type, climate, and fire in determining the distribution of *B. delagoense* in its native and introduced range – these are addressed in subsequent chapters. This chapter also links with the following three chapters which deal specifically with the biology and host range of the three agents selected for further study, as potential biocontrol agents.

The co-author Jocelyn Rajaonarison, a Madagascan entomologist, assisted with surveys in southern Madagascar – he made no contribution to the writing of this paper. The paper has been published in the journal African Entomology 12(1):1-7 (2004).

Insects associated with *Bryophyllum delagoense* (Crassulaceae) in Madagascar and prospects for biological control of this weed

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Surveys of insects associated with *Bryophyllum delagoense* in Madagascar was undertaken to identify potential biological control agents for this species in Australia. Seventy-five *B. delagoense* localities in southern Madagascar were surveyed at least once during four survey trips over a two-year period. Insects representing four orders, 15 families, and 23 species were collected, of which only 10 species were thought to be able to complete their development on *B. delagoense.* Three of these, *Osphilia tenuipes* (Curculionidae), *Rhembastus* sp. (Chrysomelidae), and *Eurytoma* sp. (Eurytomidae) were selected as potential biological control agents as they were abundant, widespread, damaging, and easy to rear.

Key words: biological control, *Bryophyllum delagoense,* insects, Madagascar, surveys.

INTRODUCTION

Bryophyllum delagoense (Ecklon & Zeyher) Schinz (Crassulaceae) (mother-of-millions, finger plant) is an erect, unbranched herb with cylindrical succulent leaves endemic to Madagascar. It has been introduced as a garden ornamental to many countries worldwide, including Australia, where, in the absence of natural enemies, it has spread over most of Queensland in the past 30 years (Hannan-Jones & Playford 2002). The plant is considered allelopathic (Hannan-Jones & Playford 2002) and consumption of its flowers by cattle has been known to cause mortality (McKenzie & Armstrong 1986). Mechanical and chemical control has largely been ineffective, with the result that Queensland Department of Natural Resources and Mines initiated a biological control programme in 1999.

There are approximately 35 genera and 1500 species in the Crassulaceae worldwide. The family has conspicuous centres of diversity in Mexico, southern and east Africa and the European and Asiatic mountains (Eggli *et al.* 1995). Many species in the Crassulaceae are native to Madagascar, with 35 species in the genus *Bryophyllum* endemic to the island (Eggli *et al.* 1995). Species within this genus have a characteristic and effective form of vegetative reproduction by means of bulbils on the edge of leaves, which establish by dropping to the ground (Tölken 1985). Four surveys for potential biological control agents were undertaken in Madagascar over a two-year period. This paper describes the insect fauna found during these surveys on *B. delagoense* and some congeners, and presents our conclusions as to the potential for some of the insect species to perform as biocontrol agents of the plant in Australia. Comparisons between our survey and similar studies are also made, with particular reference to plant architecture as one of the factors that may determine insect diversity and abundance on plant species.

MATERIAL AND METHODS

Plant surveys

Information on the distribution of *B. delagoense* and other closely related species in the genus *Bryophyllum* was obtained from a preliminary survey in Madagascar in 1997 by L.L. Dreyer, University of Cape Town, from the literature (Boiteau & Allorge-Boiteau 1995), and from a study of herbarium specimens housed at Tsimbazaza Botanical Gardens, Antananarivo. Based on this information, surveys were undertaken in southern Madagascar in August 1999, February 2000, December 2000 and June 2001. Five to 10 days were spent in the field during each survey trip. Surveys were limited to areas adjacent to roads but planned in such a way as to include most of the known distribution of *B. delagoense* in Madagascar.

Insect surveys

All plants at each locality were visually inspected for any insects or insect-related damage. Actively moving insects were collected with an aspirator, while sessile insects were removed with the plant part they were associated with. In some cases, plants were removed and the roots inspected. Several plant stems (5-15 depending on the number of plants at each site) were dissected at each locality for endophagous insects and immature stages. Plants with evidence of internal insect damage were removed whole, placed in containers, and then transferred to emergence boxes upon return to the quarantine laboratory in South Africa. All insects observed on the plant were collected, with the exception of some Orthoptera, which evaded capture.

All insect specimens were submitted to the Biosystematics Division, ARC-Plant Protection Research Institute (ARC-PPRI), in Pretoria, South Africa, for identification. Insects of which only one or two specimens were collected were generally regarded as vagrants and only identified to family level, while every attempt was made to identify more common species, which could be positively associated with *B. delagoense,* to species level. All voucher specimens are housed in the Biosystematics Division, ARC-PPRI, Pretoria, and at the Centre National de la Recherche Appliquée au Développement Rural (FOFIFA), Antananarivo, Madagascar.

RESULTS

Plant surveys

All of the 75 *B. delagoense* sites found in southern Madagascar were surveyed for insects. Most survey sites were in the central-south with the most southerly locality between Faux Cap and Cap St Marie (25°30'53"S; 45°14'59"E) and the most northerly site surveyed 3 km east of Bikitro (24°33'26"S; 45°19'30"E) (Fig. 1). The most easterly locality was north of Fort Dauphin (24°57'07"S; 46°59'23"E) with the most westerly site between Androka, near the mouth of the Linta River, and Ampanihy (24°53'47"S; 44°21'45"E) (Fig. 1). Most *B. delagoense* sites were found between Tsiombe and Antanimora and between Tranoroa and Ampanihy. No uncultivated *B. delagoense* was found in the area to the west and northwest of Ampanihy, with the exception of two field sites within 18 km of Ampanihy en route to Tulear, despite extensive surveying in this region during the flowering period in June 2001.

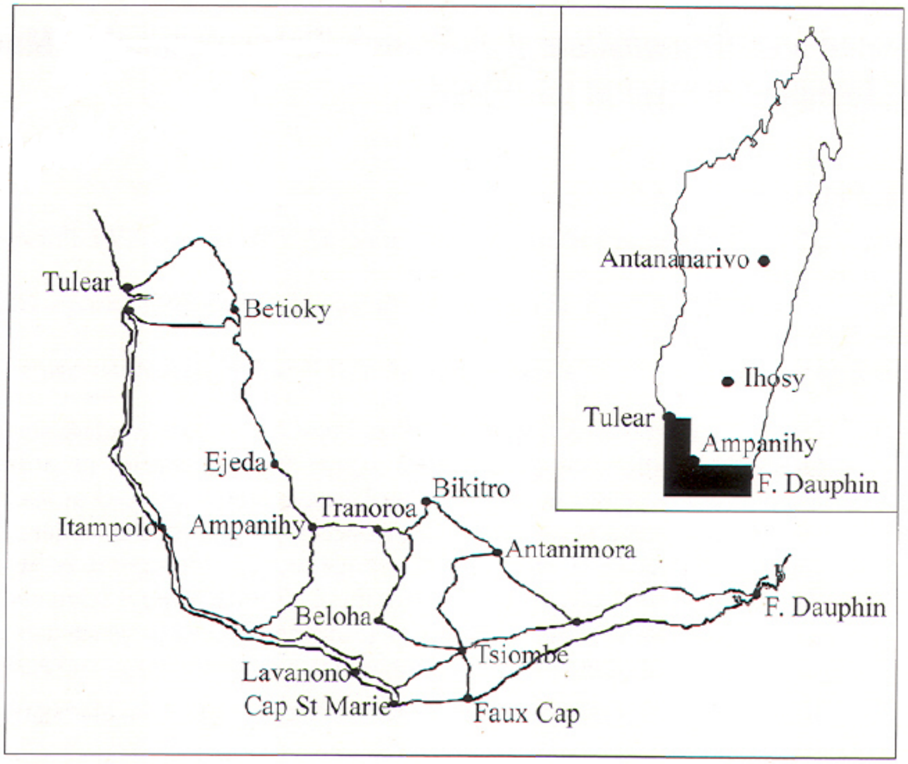

Fig. 1. Madagascar and the survey area in southern Madagascar with the routes followed during the four surveys.

The region in which *B. delagoense* was most abundant has an annual rainfall of approximately 490 mm per annum, most of which falls from November to March. Mean maximum monthly temperatures as recorded at Tsiombe range from 26.1°C in June and July to 33.3°C in December and January while July has the lowest mean minimum temperature of 11.7°C (Sutherst & Maywald 1985). Most localities were on igneous and metamorphic basement rocks, mainly granites and migmatites, and also on unconsolidated sands mainly between Beloha and Tranoroa. Plants were found growing at elevations ranging from virtually sea level to 427 m, between Andalatanosy and Antanimora (24°44’37”S; 45°35’23”E). Most sites had comparatively few adult plants (*c.* 20–250) with large numbers of seedlings (*c.* 20–2500), most of which were growing under mature plants. Plants were often found growing in association with *Xerophyta* sp., *Senecio* spp., *Pachypodium* sp., *Euphorbia* spp., *Alluaudia* spp., *Aloe* spp., and even introduced *Opuntia* spp.

Insect surveys

Coleoptera dominated the insect fauna on *B. delagoense* in southern Madagascar, while species in the Hemiptera, Lepidoptera and Hymenoptera were far less common. Twenty-three species of insects in 15 families were collected during the four surveys if one assumes that the unidentified Lepidoptera larva was from a different family to other Lepidoptera species collected (Table 1). Several grasshoppers and katydids (Orthoptera) were seen feeding on leaves but were easily alarmed and escaped before sampling and are therefore not reported. In 16 h of sampling, only eight species of insects were collected during the first survey in August 1999, with an additional 10 species in February 2000 (Fig. 2). Only seven species were collected on *B. delagoense* during the flowering period in June 2001, of which only two had never been collected in the previous three surveys.

Ten of the species of insects collected could be positively associated with *B. delagoense* in that they were either reared on the plant in the laboratory, or immature life stages were collected on the plant in the field. Five or more individual specimens were seen or collected from nine of these species during the four surveys (Table 1). These nine species had all been collected by the end of the second survey in February 2000 (Fig. 2). The remaining 13 species, with the exception of an unidentified Lepidoptera larva which died during rearing, were collected as adults and could not be associated with any feeding damage on the plant. The assumption was therefore made that they were vagrants, many of them probably not even phytophagous.

Osphilia tenuipes (Fairemaire), *Rhembastus* sp., and *Eurytoma* sp. were identified as potential biological control agents because the larvae and/or adults were generally abundant, widespread, damaging to the host plant and easy to rear. *Rhembastus* sp., *O. tenuipes* and *Eurytoma* sp. larvae, adults and/or damage were observed at 55%, 52%, and 23 % of the 75 sites surveyed, respectively.

Rhembastus sp. adults were abundant at a number of sites, especially in February, and were easily collected while feeding on the soft terminal growth. Adults also fed on the bulbils, and in June a number of adults were found feeding on flower buds and inflorescences. Feeding damage to the new growth and bulbils was characterized by a distinctive blackening on the edges of the damaged tissue. During mass-rearing in the laboratory, we found larvae of *Rhembastus* sp. feeding on the roots of *B. delagoense.*

Osphilia tenuipes adults were rarely seen and only collected on two occasions while larvae in the stems and larval feeding damage was found at a number of sites. Adult feeding damage was mainly characterized by localized feeding punctures along the stem and, in some instances, on the bulbils and leaves. Plants with larvae in their stems were weakened considerably and often broke while being inspected.

Eurytoma sp. adults were only abundant at two sites, one near Fort Dauphin (25°00’25”S; 46°54’48”E) in August and the other at a site between Tsiombe and Antanimora (25°07’03”S; 45°30’58”E) in February.

Eurytoma sp. larvae are leaf-borers and pupation occurs within the leaf. The presence of *Eurytoma* sp. at sites could therefore be confirmed by characteristic adult emergence holes in the leaves. High larval infestations resulted in leaf desiccation with many plants losing a large percentage of their leaves.

Table 1. Insects collected on *Bryophyllum delagoense* in southern Madagascar.

Species	No. of sites[a]	Stages found	Plant part
HEMIPTERA			
Cicadellidae			
Poecilocarda sp.	6	Nymphs, adults	Leaf
Eriococcidae			
Eriococcus sp.	5	Adults	Stem
Coccidae			
Unidentified coccid	3	Adults	Stem
COLEOPTERA			
Dermestidae			
Unidentified sp.*	2	Adults	Leaf
Bostrychidae			
Unidentified sp.*	1	Adults	Stem
Phalacridae			
Unidentified sp.*	1	Adults	Leaf
Coccinellidae			
Unidentified sp.*	1	Adults	Leaf
Tenebrionidae			
Unidentified sp.*	1	Adults	Leaf
Chrysomelidae			
Rhembastus (*antennatus* Jacoby) group	25	Adults	Bulbil, leaf, flower
Glaucorhabda madagascariensis Jacoby*	1	Adults	Leaf
Curculionidae			
Osphilia tenuipes (Fairmaire)	18	Larvae, adults	Stem, leaf
?*Catalalus* sp.*	2	Adults	Leaf
Genus nr.?*Catalalus* sp.*	1	Adults	Leaf
?*Proictes* sp. 1*	3	Adults	Leaf
?*Proictes* sp. 2*	1	Adults	Leaf
?*Proictes* sp. 3*	1	Adults	Leaf
LEPIDOPTERA			
Psychidae			
Acanthopsyche sp.	9	Larvae	Leaf
Typhonia sp.	12	Larvae	Leaf

Species	No. of sites[a]	Stages found	Plant part
Unidentified sp.*	1	Larvae	Leaf
Lycaenidae			
Leptomyrina phidias Fabricius	17	Larvae, pupae	Leaf, flower
Noctuidae			
Agrotis longidentifera ranavalo Viette*	1	Larvae	Leaf
Unidentified Lepidoptera[+],*	1	Larvae	Leaf
HYMENOPTERA			
Eurytomidae			
Eurytoma bryophylli	9	Larvae, adults	Leaf

[+] Died as larva.

*Fewer then five individuals seen.

[a] Number of sites where larvae, nymphs and/or adults were seen.

These three species were not seen on any other of the four species in the genus *Bryophyllum* surveyed in Madagascar. However, it should be noted that few localities supporting other *Bryophyllum* species were found in the area surveyed in southern Madagascar. Despite an intensive search for *B. daigremontianum* (Hamet & Perrier) in areas where it had previously been recorded, it was found only in domestic gardens, while *B. proliferum* (Bowie) Hamet and *B. pinnatum* (Lam.) Persoon were found at two localities to the north of Fort Dauphin. *Leptomyrina phidias* (Fabricius) (Lycaenidae) was the only insect species collected on *B. delagoense,* that was also found on all of the other *Bryophyllum* species surveyed. *Osphilia tenuipes* larvae were also collected from the stems of *Kalanchoe pubescens* Baker (non R. Br. ex Britt.) near Fort Dauphin. *Aphthona* sp. (Chrysomelidae: Alticinae) was a particularly abundant beetle found feeding on the leaves of *B. proliferum, B. pinnatum,* and *B. fedtschenkoi* Hamet & Perrier, but was not collected on *B. delagoense* during the survey.

DISCUSSION

Extensive literature searches have revealed that no complete surveys for insects or pathogens have been undertaken on any species in the Crassulaceae worldwide.

However, there is a record of *Alcidodes sedi* (Marshall) (Coleoptera: Curculionidae) reared from stems of a *Sedum* (= *Crassula,* see Tölken 1985) species in Pretoria, South Africa (Marshall 1938). A number of species in the family Lycaenidae (Lepidoptera) have also been reared and collected on native and introduced species in the family Crassulaceae in southern Africa (Dickson 1947; Kroon 1976, 1999; Pringle *et al.* 1994). There are some records of *Phytomyza* spp. (Diptera: Agromyzidae) on *Rhodiola* and *Sedum* spp. in North America (Griffiths 1976), *Cheilosia* spp. (Diptera: Syrphidae) on *Sempervivum* and *Sedum* spp. in Europe (Schmid 2000) and *Callirhopalus bifasciatus* (Roelofs) (Coleoptera: Curculionidae) on a *Sedum* sp. in the United States (Marrone & Zepp 1979). None of these give any indication of the total number of insect species collected (Kooi 1988; Skaria *et al.* 1997; Kroon 1999). The only complete survey on a species in a closely related family (Leistner 2000) was undertaken from 1967–1969 in Yugoslavia on an aquatic floating plant *Myriophyllum spicatum* L. (Haloragaceae) (Lekić & Mihajlović 1970).

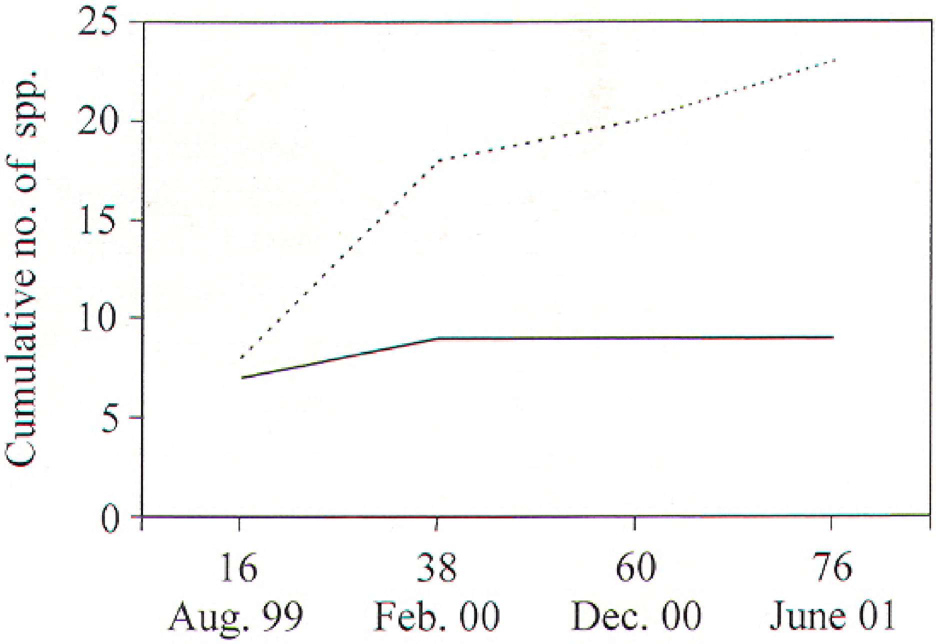

Fig. 2. Species accumulation curves showing all of the species (dotted line) and the species of which more than five specimens (solid line) were collected during four surveys in Madagascar.

Approximately 15 species of insects were collected, of which only two, *Parapoynx strationata* L. (Lepidoptera: Pyraustidae) and *Litodactylus leucogaster* Marsh (Coleoptera: Curculionidae), were considered as potential biological control agents (Lekić & Mihajlović 1970). Although the total insect fauna on *M. spicatum* is comparable to what we found on *B. delagoense,* it is difficult to compare insect faunas on submerged aquatic and terrestrial plants.

Compared to other plant species that have been surveyed extensively for phytophagous insects (Goeden 1971; Goeden 1974; Goeden & Ricker 1976, 1986), few insect species were collected on *B. delagoense* The dearth of insects can probably be attributed to the architectural simplicity of the plant (Strong *et al.* 1984), its limited geographical distribution (Strong 1979), and the fact that it occurs in an arid region. Architecturally complex plants potentially have more niches and provide more enemy-free space, and are therefore expected to support a greater number of insect species (Moran 1980; Strong *et al.* 1984). Morphologically, *B. delagoense* is a simple plant with succulent, cylindrical leaves and a smooth stem, characteristics that have probably evolved primarily as an adaptation to a xerophytic habit. However, an additional selective force for a reduction in plant structural complexity may have come from the insect community itself, as a simpler plant structure has been proposed to lead to a reduction in insect herbivory (Moran 1980). Surveys of 13 *Opuntia* species in their native range found that the small, narrow-jointed species such as *O. salmiana* Parm. each support on average only nine species of phytophagous insects compared to the large tree-like opuntias which each support about 16 species (Moran 1980). From our surveys, we concluded that *B. delagoense* only supports 10 species of phytophagous insects, which is similar to that found on small *Opuntia* species. Although the Cactaceae is distantly related to the Crassulaceae, *Opuntia* species are similar in many respects in that they are succulents, architecturally simple, grow in arid regions and many of the smaller species have limited geographical ranges (Moran 1980). One could therefore conclude that the number of species of insects collected on *B. delagoense* is probably an accurate reflection of the total number of species that occur on this plant in southern Madagascar; this is supported by the asymptotic species accumulation curves (Fig. 2).

Further evidence to support our contention that the majority of insect species on this plant have been collected, can be drawn from the fact that we collected insects feeding on all of the perceived niches available on the plant. However, we did not find any insect species, other than those we had found feeding on other plant parts, feeding specifically on the flowers, buds and seeds. A few *Rhembastus* sp. adults and *L. phidias* larvae, which normally feed on the leaves, were found feeding on flower buds and flowers in June. Most species of insects collected were leaf-feeders, with only *O. tenuipes* feeding on the stems, and *Rhembastus* sp. larvae on the roots.

Two of the species selected, *O. tenuipes* and *Eurytoma* sp., have endophagous larvae while *Rhembastus* sp. larvae are root-feeders. These life-history strategies may protect them from generalist predators and parasitoids in Australia. Based on field surveys it is clear that *O. tenuipes* is not monophagous. However, this should not preclude it as a potential agent at this early stage as there are no native *Kalanchoe* species in Australia. All three species are currently undergoing host range testing in quarantine facilities in South Africa and Australia.

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CHAPTER 4

INITIAL SCREENING OF THE STEM-BORING WEEVIL *OSPHILIA TENUIPES*, A CANDIDATE AGENT FOR THE BIOLOGICAL CONTROL OF *BRYOPHYLLUM DELAGOENSE* IN AUSTRALIA

Justification

Successful biocontrol depends on the damage each individual agent can inflict on the target plant; the ecology of the agent, which determines the population density achieved in the new environment; and the ecology of the weed, which determines whether that total damage is significant in reducing the population (Cullen, 1995). Biocontrol agents are therefore selected based on a number of traits, the most important being that they should have the ability to build-up populations rapidly in the country of introduction (Crawley, 1988), be widespread and abundant in the country of origin, be damaging and have a narrow host range (Crawley, 1986; Goeden, 1983). Agents with multiple generations per year will be favoured because they can build-up populations more rapidly than univoltine insects (Goeden, 1983), clearly a very beneficial attribute. However, even multivoltine insects may fail to build-up populations rapidly or even establish because of their interaction with environmental factors such as climate and parasitoids, predators and competitors in the country of introduction (Blossey, 1995a; Wapshere, 1985). Climatic tolerance can possibly be predicted from the distribution of the agent in the country of origin; an agent with a wide distribution may be better adapted to a broader range of climatic conditions compared to an agent with an extremely restricted distribution (Goeden, 1983).

The damage caused by the agent is best determined under laboratory conditions, supported by field observations (McFadyen, 1998; Schroeder and Goeden, 1986; Sheppard *et al*., 2003). The host specificity of a potential biocontrol agent can be determined from field surveys in the country of origin, but this is usually only used as supporting evidence for laboratory trials, as not all potential hosts are present in the country of origin. However, many agents often exhibit a wider host range in laboratory trials than they would under natural conditions in the field (McFadyen, 1998). Such feeding may be an artifact of confinement, whereby restriction in cages prevents normal host-finding behaviour (Harris and McEvoy, 1995: Louda *et al*., 2003) and can possibly be resolved by using larger cages (Cullen, 1990).

This chapter contributes to the body of literature on classical biological control by providing information on the biology, host range and impact of a new potential biocontrol agent for *B. delagoense*. We consider some of the attributes of the stem-boring weevil, *Osphilia tenuipes*, to ascertain if it has potential as a biological control agent for *B. delagoense* in Australia. It follows on from the preceding chapter which looked at the natural enemies associated with *B. delagoense* in Madagascar, of which *O. tenuipes* was identified as one. Based on preliminary host range trials *O. tenuipes* would appear to have potential as a biocontrol agent in Australia although its ability to develop on *Kalanchoe* species may mitigate against its use in regions where this genus is present. Australia has only eight indigenous species of Crassulaceae, all belonging to the genus *Crassula* (Tölken, 1981). *Crassula* species did support the development of *O. tenuipes* in preliminary host range trials.

The weevils' short generation time and its ability to inflict heavy damage on its host are additional traits which contribute to its potential as an agent. The fact that *O. tenuipes* was present throughout the range of *B. delagoense* in southern Madagascar indicates that it may be tolerant of a wide range of climatic conditions, another beneficial trait (Goeden, 1983; Harris, 1973). It leads to the next chapter which investigates the potential of a wasp, *E. bryophylli*, as an additional biocontrol agent to compliment *O. tenuipes* should it ever be released in Australia.

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Initial screening of the stem-boring weevil *Osphilia tenuipes*, a candidate agent for the biological control of *Bryophyllum delagoense* in Australia

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Abstract. A stem-boring weevil, *Osphilia tenuipes* (Fairmaire) (Coleoptera: Curculionidae), from Madagascar, was screened in South Africa to determine its potential as a biological control agent for *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz (Crassulaceae) in Australia. Favourable attributes of the weevil include ease of culturing, multiple generations per year, and high levels of damage inflicted on *B. delagoense* under laboratory conditions. Despite indications that *O. tenuipes* has a narrow field host range, no-choice and multiple choice trials in quarantine revealed that it could oviposit and develop to adulthood on seven non-target species in the family Crassulaceae, without an obvious loss of fitness on four of those species. Despite these results, *O. tenuipes* is considered to have potential for release against *B. delagoense* in Australia because the continent has very few native Crassulaceae which could potentially serve as alternative hosts in the field.

Key words: biology, *Bryophyllum delagoense*, Curculionidae, host range, *Osphilia tenuipes*, weed biocontrol

Introduction

Bryophyllum delagoense (Ecklon & Zeyher) Schinz (Crassulaceae, 'mother-of-millions'), native to Madagascar, is a serious weed in Queensland, Australia. The plant has invaded thousands of hectares of grazing land and is poisonous to livestock as it contains bufadienolide glycosides (Capon et al., 1986). Invasion is rapid because each flower head produces thousands of seeds and each leaf promotes vegetative spreading by producing new plantlets, which establish by dropping to the ground. Established infestations are difficult and expensive to control using chemical or mechanical means (Hannan-Jones and Playford, 2002), and because this plant has few natural enemies in Australia (Hannan-Jones and Playford, 2002) biological control is considered to be a necessity.

Osphilia tenuipes (Fairmaire) (Coleoptera: Curculionidae: Conoderinae) (previously Zygopinae: see Alonso-Zarazaga and Lyal, 1999) was collected in Madagascar and appears to be a promising agent for the control of *B. delagoense*. The adults feed externally on the stems, and occasionally on the leaves and plantlets, while the larvae are stem-borers. Extensive feeding by adults and larvae weakens plants considerably and often results in stem breakage. Weevils have been used extensively in the biocontrol of weeds worldwide (Julien and Griffiths, 1998). Many species are either monophagous or oligophagous and are able to cause considerable damage. This paper reports on some aspects of the biology and preliminary host range testing of *O. tenuipes* in South Africa prior to shipment for further testing in Australia.

Materials and methods

Field observations

The distribution of *O. tenuipes* was determined by examining different species of Crassulaceae, in the natural range of *B. delagoense*, in southern Madagascar (Figure 1) for the presence of feeding damage and/or adults. Larvae in stems were collected and reared to adulthood to permit confirmation of their identity. *Kalanchoe linearifolia* Drake and *K. beharensis* Drake growing in association with *B. delagoense* were each surveyed at more than five localities. *Bryophyllum pubescens* Baker, *B. fedtschenkoi* Hamet & Perrier, *B. pinnatum* (Lamarck) Persoon, *B. proliferum* (Bowie) Hamet and *B. daigremontianum* Hamet & Perrier were only examined at two or three localities (Table 1). These species generally do not occur sympatrically with *B. delagoense*, other than in cultivated situations.

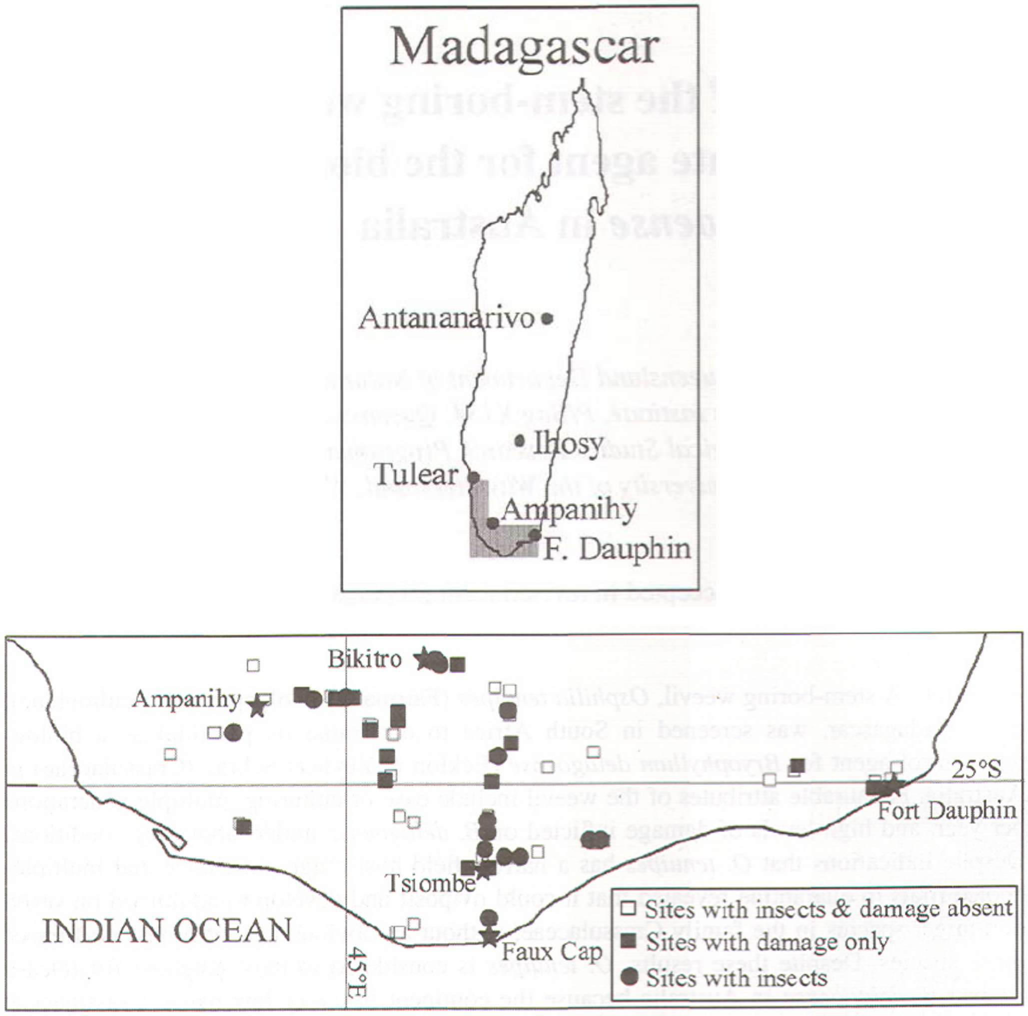

Figure 1. General survey area in southern Madagascar (top, shaded). Specific localities where *Bryophyllum delagoense* was surveyed, indicating where *Osphilia tenuipes* damage was evident and where adults or larvae were recovered (bottom).

Plants at the various field sites were surveyed for insects and pathogens during four separate trips to Madagascar in August 1999, February and December 2000 and in June 2001 (Witt and Rajaonarison, 2004). During each trip an average of approximately 70 individual plants were surveyed at each site. Observations on the phenology of *O. tenuipes* were made by recording the presence of adults on the plants and larvae in the stems during these surveys. Natural enemies of *O. tenuipes* were also recorded. Levels of parasitism were determined by counting the number of parasitoids emerging from larvae in stems collected during the survey in August 1999.

Table 1. Plant species in the Crassulaceae surveyed in Madagascar for the presence of *Osphilia tenuipes*

Species	Localities surveyed	Occasions[a]	*O. tenuipes* present
Bryophyllum delagoense	75	1-4	32
B. pubescens	2	1	1
B. pinnatum	2	1	0
B. proliferum	2	1	0
B. daigremontianum	3	1	0
B. fedtschenkoi	2	1	0
Kalanchoe linearifolia	6	2	0
K. beharensis	11	2	0

[a] Number of occasions each locality was surveyed.

Laboratory cultures and biological studies

Stems containing *O. tenuipes* larvae were collected in Tulear Province, southern Madagascar, in August 1999, and imported into South Africa. The infested stems were placed in emergence boxes in the quarantine facility of the ARC-Plant Protection Research Institute (ARC-PPRI) in Pretoria. Newly emerged adults were placed in large cages containing potted *B. delagoense* plants, which were maintained in a quarantine glasshouse under natural light at approximately 25◦C and a relative humidity of about 52%. Adults were randomly removed from this culture and used in biology studies and host specificity tests as required. The sex ratio of 50 adults collected from this culture was 1:0.72 (females: males) with 70% of the females gravid. It was therefore assumed that any adults used in trials would virtually have an equal number of males and females. The number of adults emerging from stems in various size categories during mass rearing was recorded by counting the number of emergence holes on each stem (Table 2). The number of emergence holes corresponded to the number of adults in cages during mass rearing. Voucher specimens of the weevil have been lodged with the National Collection of Insects (ARC-PPRI, Pretoria, South Africa) and at the Centre National de la Recherche Appliquée au Développement Rural (FOFIFA, Antananarivo, Madagascar).

The development of immature stages was studied by placing 15 adults in each of 36 plastic sleeves placed on potted *B. delagoense* for 24 h. Each sleeve consisted of an empty two-litre plastic bottle with the bottom end removed. The bottom end was stoppered with a high-density foam disc with a slit to allow for the placement of the plant stem. Gauze covered openings in the sleeves provided ventilation. Approximately 160 mm of each plant stem was available to the weevils. Six sleeved plants were dissected on each day, 5, 10, 20, 30, 40 and 50 days after the adults had been removed. The number and size of the eggs, larvae, pupae and adults that were found in the dissected stems were recorded using an ocular micrometer. The data on larval head capsule widths were analysed using cluster analysis (Payne et al., 1989). Non-hierarchial clustering was used to determine the number of instars. The criterion used for determining the optimum classification was the minimum within-group sum of square's method. After the optimum classification was obtained an analysis of variance was used to test for differences between the instars. Instar means were separated using the Fishers' protected t-test (Snedecor and Cochran, 1980).

Relative survival of *O. tenuipes* adults was assessed for insects deprived of food. The survival of adults in sleeves containing *B. delagoense* stems and moist florists' foam was compared with that of adults kept in sleeves containing either only dry florists' foam or only moist florists' foam. One weevil was placed in each of 9 sleeves used for each of the three treatments, and survival was monitored every 48 h until the last adult in the treatments devoid of plant material had died.

Adult longevity was determined by placing a mating pair of newly emerged (within 24 h) adults in each of eight plastic sleeves on a potted plant. Each pair of adults was placed on a fresh plant every seven days. Sleeved plants were placed in a constant environment room which had a mean (± SE) day and night temperature of 28 ± 2^{o}C and 23 ± 2^{o}C respectively and a relative humidity of 55 ± 20%.

Host-specificity tests

No-choice trials. These tests were undertaken to determine the laboratory host range of *O. tenuipes*. Adults were exposed to 15 test species, including 12 species in the family Crassulaceae, two in the Lamiaceae and one in the Portulacaceae (Table 3). These species were tested because they are either taxonomically related to *B. delagoense* or are similar morphologically, by having succulent stems. In each trial ten randomly collected adults were placed in each of six cages (650 mm × 400 mm × 430 mm). Five cages each had a single test plant and one cage contained *B. delagoense* as a control. Six trials were completed, with the result that each plant species was tested twice. Trials were conducted in the quarantine laboratory under natural light, with temperatures ranging from 21 to 26^{o}C. Mean relative humidity during the day was approximately 50% and at night about 55%.

After 20 days, all adults were removed and the number of live adults recorded. Feeding damage was then categorized on an absence/presence basis. Plants used in each trial were then retained in their cages and monitored daily for adult emergence. Monitoring of all plants ceased two weeks after the last adults had emerged from the *B. delagoense* controls.

Multiple choice trials. In no-choice trials potential biological control agents often exhibit a wider host range than would normally occur in the field. To determine whether these results could have been due to aberrant host selection behaviour, multiple choice tests were undertaken in large cages (900 mm × 860 mm × 475 mm). In each cage, plants of five non-target species plus *B. delagoense* were exposed to *O. tenuipes*. Thirty adults were released into each cage and removed after 10 days. The number of feeding punctures on each plant were counted and the plants were monitored daily for adult emergence. Monitoring ceased two weeks after the last adults had emerged from *B. delagoense*. One or two plants of each of the 11 non-target species, were tested in this way (Table 4).

Results

Field observations

Osphilia tenuipes larvae and/or adults were observed, or damage, which was distinct, was recorded, at 32 of the 75 sites where *B. delagoense* was surveyed in southern Madagascar (Figure 1). *Osphilia tenuipes* was also found on one other species besides *B. delagoense* in Madagascar. Larvae were reared from *B. pubescens*, which was sampled at Pic St. Louis (25^{o}00’40”S, 46^{o}58’08”E) near Fort Dauphin. No adults or larvae were recovered on any of the other related species that were surveyed (Table 1).

Based on the surveys, the weevil has a wide distribution from south-west of Ampanihy (24^{o}48’36”S, 44^{o}35’17”E; 236 m) to Fort Dauphin (25^{o}00’40”S, 46^{o}58’08”E; 398 m) in the east and near Bekitro (24^{o}33’26”S; 45^{o}19’30”E; 244 m) in the north to slightly north (25^{o}29’33”S, 45^{o}31’03”E; 233 m) of Faux Cap in the south (Figure 1). A number of dead plants at each of these sites had hollowed stems with characteristic emergence holes indicative of *O. tenuipes* damage. During surveys in June and December, larvae were recovered in the stems and adults were seen on the plants. Athough adults were recorded during the February survey, no larvae were recovered at this time. Only larvae and no adults were recovered during the August survey. This would suggest that *O. tenuipes* has multiple generations per year in the field. Of the 48 larvae that were collected in the field in August 1999 and reared in the laboratory, 11 (23%) were parasitized.

Two *Oxysychus* species (Hymenoptera: Pteromalidae) emerged from stems containing *O. tenuipes* larvae.

Biology of Osphilia tenuipes

Thirty-seven *O. tenuipes* adults emerged from 41 infested stems collected from nine localities in southern Madagascar in August 1999. Adults, measured from the mid-anterior edge of the pronotum to the posterior part of the elytra, were 2.61 ± 0.23 mm (mean ± SE; $n = 10$) long with no discernable external differences between males and females. Adults are brown with black longitudinal stripes on the thorax and elytra, and have large compound eyes and a slender rostrum.

Soon after emergence, adults started feeding on the stem and, to a lesser extent, on the leaves and plantlets. Feeding punctures were clearly visible within localized areas along the stem. This localized feeding resulted in the desiccation of plant tissue, which weakened the stem considerably. Adults copulated within 24 h after emergence and females oviposited in the feeding holes. Larval tunnelling compounded the weakening of the stem, with plants initially dropping leaves and plantlets, after which the stem often dried out completely. Large numbers of adults emerged from infested stems, with up to 25 emergence holes recorded from a single stem in the 300–399 mm length category (Table 2). The number of emergence holes tended to increase in longer stems (Table 2). These were not thicker than shorter stems as indicated by the Pearson's coefficient of correlation which showed a weak correlation between stem length and diameter ($n = 33$; $r = 0.3440$; $P < 0.05$) (Snedecor and Cochran, 1980).

Stems dissected only five days after having been exposed to adults contained both eggs and larvae (Figure 2), indicating that the egg incubation period, in some cases, can be approximately five days or less at a mean (± SE) day and night temperature of 28 ± 2^{o}C and 23 ± 2^{o}C, respectively, and a relative humidity of 55 ± 20%. The pale yellow eggs are deposited in feeding holes along the stem and have a mean (± SE) length of 0.520 ± 0.002 mm and a mean (± SE) width of 0.320 ± 0.001 mm ($n = 121$).

Table 2. Number of adult *Osphilia tenuipes* emerging from *Bryophyllum delagoense* stems in various length categories from the laboratory culture

Length category (mm)	*n*[a]	Emergence holes[b]	Range[c]
100–199	19	3.26 ± 0.69	0-12
200–299	23	5.08 ± 0.69	0–11
300–399	4	11.25 ± 4.87	3–25
400–499	4	8.25 ± 4.30	1–18
500–599	1	24	—

[a] Number of stems.

[b] Mean number of emergence holes with standard errors.

[c] Range in number of emergence holes for all stems evaluated in each length category

Analysis of the head capsule width measurements suggested that *O. tenuipes* has five larval instars (Figure 3). First instar larvae, some of which were dissected from stems five days after adult exposure, had a mean (± SE) head capsule width of 0.220 ± 0.000 mm (*n* = 22), and were found directly beneath the adult feeding lesions. Final instar larvae had a mean (± SE) head capsule width of 0.100 ± 0.009 mm (*n* = 52). The apodous larvae are C-shaped and are creamy-white with a very distinct brown head capsule. Each larva mines approximately 5 cm in the stem before pupating in a cavity within the stem. The first pupae were dissected from stems 40 days after adult exposure, at which time adults had also emerged from plants (Figure 2). These observations indicate that *O. tenuipes* takes approximately 40 days to develop from egg to adulthood.

All *O. tenuipes* adults deprived of *B. delagoense* stems died within ten days (Figure 4). Adults with access to water survived in higher proportions than those deprived of water, but did not survive longer (Figure 4). More than 80% of adults with access to living stems were still alive after 10 days.

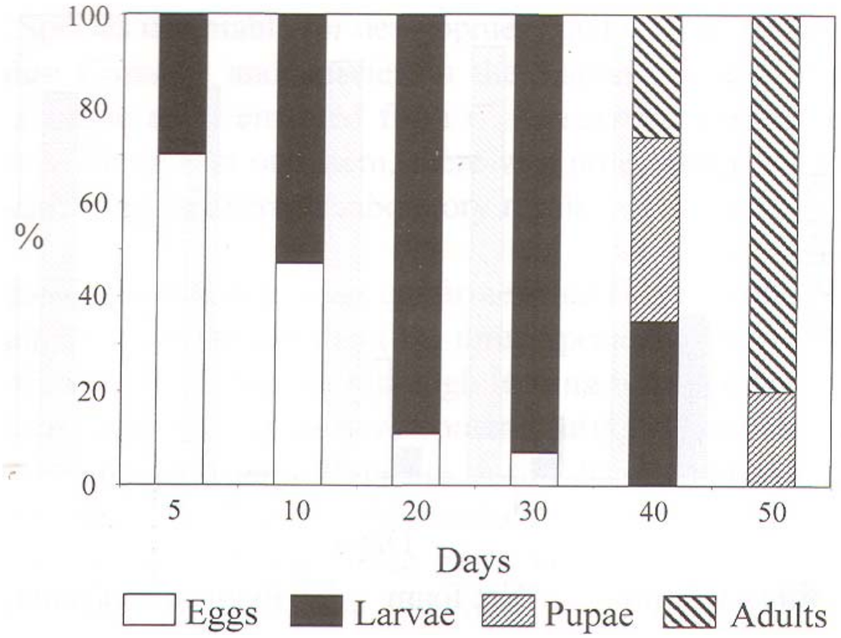

Figure 2. Percentages of eggs, larvae and pupae of *Osphilia tenuipes* found in *Bryophyllum delagoense* stems which were dissected 5, 10, 20, 30, 40 and 50 days (*n* = 6 for each time period) after exposure to 15 adults for 24 hours at a mean (± SE) day and night temperature of 28 ± 2°C and 23 ± 2°C respectively and a relative humidity of 55 ± 20%. The percentage of adults that emerged is also shown.

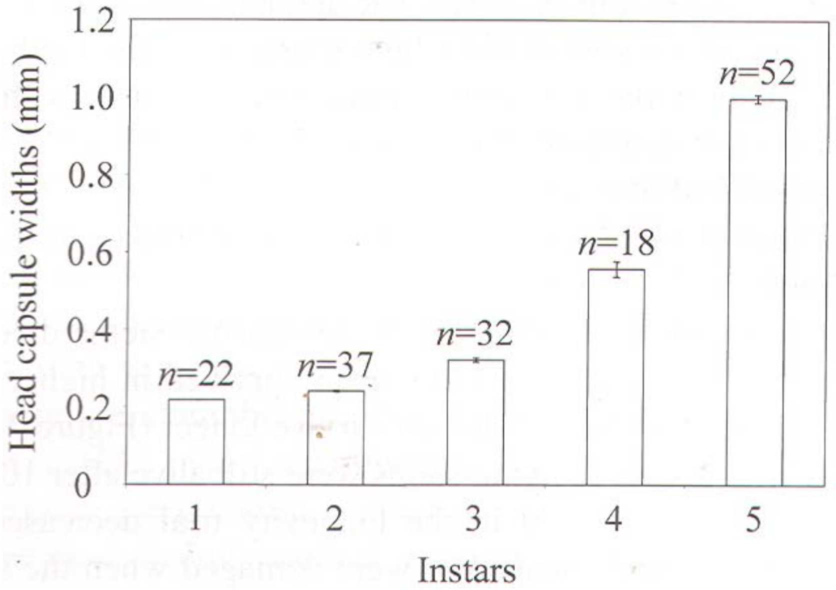

Figure 3. Mean (± SE) head capsule widths of the five *Osphilia tenuipes* larval instars.

The number of adults used in the longevity trial decreased over time because the weevils either escaped or were damaged when the sleeves were placed on fresh potted plants every seven days. Only seven of the 18 adults used in this trial died of perceived natural causes after an average (± SE) of 65.3 ± 9.4 (*n* = 7) (range = 35–99) days. One adult escaped after 121 days.

Host specificity of O. tenuipes

No-choice trials. Out of the 15 non-target species tested no-choice trials indicated that *O. tenuipes* could feed on six species and complete larval development on four species (all in the family Crassulaceae) (Table 3). Successful oviposition and development occurred in *B. daigremontianum*, *B. fedtschenkoi*, *K. sexangularis* N. E. Br. and *Crassula sarcocaulis* Ecklon & Zeyher. Species unsuitable for development included all remaining species in the genus *Crassula* and species in the Lamiaceae and Portulacaceae. Although a single adult emerged from *C. sarcocaulis* and there was some larval feeding damage in one stem, there was no evidence of adult feeding damage, suggesting an aberrant laboratory result.

Multiple-choice trials. These trials confirmed that *O. tenuipes* fed, oviposited on and completed its development on three species of *Bryophyllum* and a species of *Kalanchoe* (Table 4). Although feeding was recorded on *K. sexangularis* which supported larval development during the no-choice trials, no adults were recovered from this species during these trials. Similarly, larval development was noted *on C. sarcocaulis* in the no-choice trials but the plant was not attacked in the choice trials. However, oviposition occurred and a single adult emerged from *Echeveria* sp. A (Crassulaceae), despite this species not supporting oviposition during the no-choice trials. This could also be a result of aberrant host selection behaviour of females in laboratory situations.

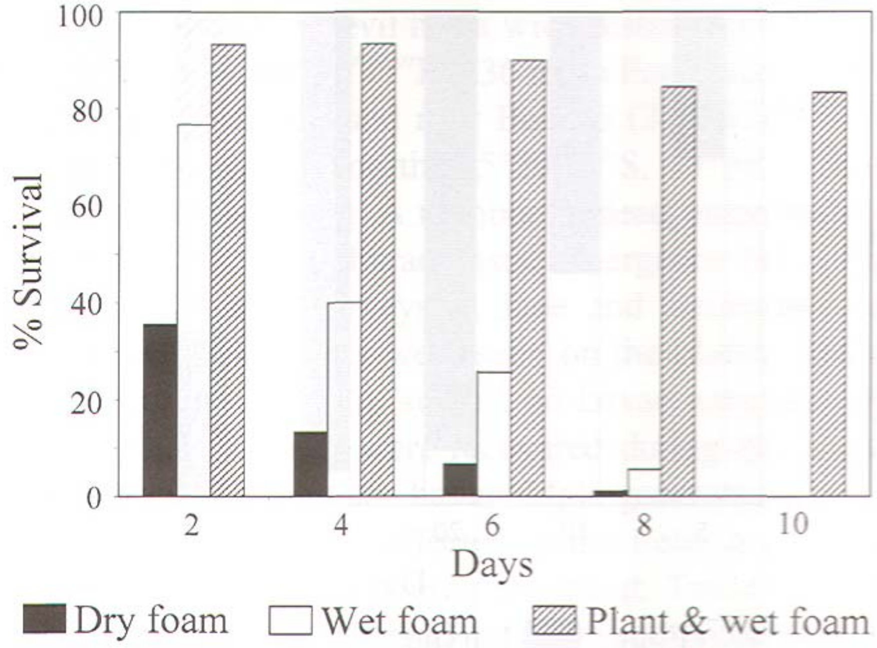

Figure 4. Percentage of *Osphilia tenuipes* adults that survived in sleeves containing either dry florists' foam only (n = 9), wet florists' foam only (n = 9), or growing plants with wet florists' foam (n = 9).

Discussion

There are 214 genera in the subfamily Conoderinae (previously Zygopinae) distributed worldwide, mainly in the tropics (Alonso-Zarazaga and Lyal, 1999). Hustache (1934, 1956) catalogued 49 species in the genus *Osphilia*, 14 are native to Madagascar and the remainder from tropical Africa and south-east Asia. Most *Osphilia* species have been recorded from dead or dying wood, with very few records of any species feeding on living tissue. However, there are a number of other conoderine species whose larvae are seed-feeders (C.H.C. Lyal, Department of Entomology, The Natural History Museum, London, pers. comm.). Other species have been collected in forest leaf litter and most recently from *B. pinnatum* in South Africa (A.B.R. Witt, pers. obser.).

Table 3. Laboratory host range of *Osphilia tenuipes* as determined by adult and larval survival on different test species during no-choice tests

Test plants	*n*[a]	Adults alive (mean ± SE)	Adults emerged (mean ± SE)	Days to emergence (mean ± SE)	Feeding damage
Crassulaceae					
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz*	6	8.0±1	3.0± 1	45.2 ± 1.1	Yes
B. daigremontianum Hamet & Perrier*	2	5.5± 0.5	1.5 ± 0.5	47.7 ± 0.3	Yes
B. fedtschenkoi Hamet & Perrier*	2	2.0±1	4.5± 3.5	49.8 ± 0.4	Yes
Kalanchoe sexangularis N.E.Br.*	2	3.0±1	5.5± 3.5	48.8 ± 0.6	Yes
K. orgyalis Baker*	2	4.5± 0.5	0	—	Yes
K. beharensis Drake*	2	0	0	—	No
Crassula sarcocaulis Eckl. & Zeyh.	2	0	0.5±1	61	No
C. ovata (Mill.) Druce	2	0	0	—	No
C. swaziensis Schönland	2	0	0	—	No
C. rupestris Thunb.	2	0	0	—	No
C. sarmentosa Harv.	2	3.0 ±2	0	—	Yes
Crassula sp. A	2	0	0	—	No
Echeveria sp. A*	2	0	0	—	Yes
Lamiaceae					
Plectranthus cylindraceus Hochst. Ex Benth.	2	0	0	—	No
P. tetensus (Bak.)	2	0	0	—	No
Portulacaceae					
Portulacaria afra Jacq.	2	0	0	—	No

*Species not native to southern Africa excluding Madagascar.

[a] Number of replicates.

Table 4. Host selection of *Osphilia tenuipes* as determined by their feeding, oviposition and larval development on different test species during multiple-choice tests

Test plants	n[a]	Feeding holes (mean ± SE)	Adults emerged (mean ± SE)	Days to emergence (mean ± SE)
Crassulaceae				
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz*	4	478.0 ± 75	12.3 ± 4.3	48.9 ± 0.6
B. proliferum (Bowie) Hamet*	2	233.0 ± 47	12.5 ± 1.5	57.0 ± 0.7
B. daigremontianum Hamet & Perrier*	2	551.0 ± 291	23.5 ± 21.5	48.7 ± 0.5
B. fedtschenkoi Hamet & Perrier*	2	93.0 ± 8	13.5 ± 2.5	55.0 ± 0.7
Kalanchoe sexangularis N.E.Br.*	2	107.5 ± 15.5	0	—
K. blossfeldiana (von Poellnitz) Boiteau*	2	48.5 ± 11.5	4.0 ± 2	43.1 ± 1.2
K. rhombopillosa Mannoni & Boiteau*	1	0	0	—
Crassula sarcocaulis Eckl. & Zeyh.	2	0	0	—
Crassula sp. A	2	0	0	—
Echeveria sp. A*	2	144.0 ± 144	0.5 ±1	52
Lamiaceae				
Plectranthus tetensus (Bak.)	1	0	0	—
Portulacaceae				
Portulacaria afra Jacq.	2	0	0	—

*Species not native to southern Africa excluding Madagascar.

[a] Number of replicates.

Most studies on the biology of the Conoderinae have been limited to important pests like the spotted sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte). Its larvae are very similar to those of *O. tenuipes* in that they feed inside stems of sunflowers (Charlet, 1983). Adult *C. adspersus* feed on stem and leaf tissue and lay their eggs under the stem epidermis. Larvae also feed in the vascular tissue and pith. At high larval population levels they can cause stem breakage and loss of the entire sunflower head prior to harvesting (Charlet, 1983). *Osphilia tenuipes* larvae inflicted similar damage in that they caused stem breakage of *B. delagoense* in Madagascar and the laboratory. In less severe infestations, *C. adspersus* larvae cause lower sunflower yields by interfering with the transport of nutrients through the destruction of the xylem and phloem tissues (Charlet, 1983). Although no studies were undertaken on the impact of *O. tenuipes* larvae on the flowering or seed production of *B. delagoense*, it is likely that its impact will be similar to *C. adspersus* on sunflowers.

Many curculionid species are monophagous or oligophagous and have thus featured extensively in the biological control of weeds (Julien and Griffiths, 1998). Preliminary results suggest that *O. tenuipes* is oligophagous in that larvae can complete their development on only a few closely related species of Crassulaceae. Two other species of Conoderinae have also been considered as biological control agents but were not pursued due to their lack of host specificity. *Cylindrocopturus adspersus* was apparently considered for the biocontrol of cocklebur (*Xanthium* spp.: Asteraceae) in Australia, but later rejected as a potential agent (H.A. Hespenheide, Department of Organismic Biology, Ecology and Evolution, University of California, USA, pers. comm.). Similarly, *Cylindrocopturus armatus* Champion from Mexico was considered as a possible agent for the biological control of parthenium weed, *Parthenium hysterophorus* L. (Asteraceae), also in Australia, but rejected after host range trials (H.A. Hespenheide, personal communication). These considerations suggest that conoderine weevils have potential as weed biocontrol agents if their host range is sufficiently narrow.

Despite observations that *O. tenuipes* can complete its development on a few non-target species of Crassulaceae, it can still be considered for release in Australia. The genus *Crassula* in Australia is represented by only eight native but many exotic species. (Tölken, 1981). *Kalanchoe crenata* has been recorded from north-western Western Australia, but it is unclear whether this species is native or introduced to Australia (Kenneally, 1983). Western Australian populations of *K. crenata* are geographically isolated from infestations of *B. delagoense*, which are mostly in Queensland. The possibility of attacks on native Australian Crassulaceae seems remote since *O. tenuipes* did not accept South African species of *Crassula* during no-choice tests. In addition, native Australian *Crassula* species are small plants, much smaller than *B. delagoense* and are unlikely to have sufficient stem material to support larval development. This has been shown in preliminary host range trials in Australia (W.A. Palmer, Alan Fletcher Research Station, Brisbane, Australia, pers. comm.) where larvae could not complete their development on any of the native *Crassula* species tested. More intensive tests will have to be carried out in Australia on these native Crassulaceae species and related non-native horticultural plants before this agent can be considered safe for release. However, the high levels of damage inflicted by *O. tenuipes* on *B. delagoense* and the possibility that the weevil may escape attack by native parasitoids in Australia because of the endophytic habit of the larvae makes it an excellent candidate for biocontrol.

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CHAPTER 5

DISTRIBUTION AND ASPECTS OF THE BIOLOGY AND HOST RANGE OF *EURYTOMA* SP. (HYMENOPTERA: EURYTOMIDAE), A CANDIDATE AGENT FOR THE BIOLOGICAL CONTROL OF *BRYOPHYLLUM DELAGOENSE* (ECKLON & ZEYHER) SCHINZ (CRASSULACEAE) IN AUSTRALIA

Justification

This chapter is similar to the preceding one in that it evaluates the potential of another insect, *E. bryophylli*, as a biological control agent. Please note that this insect has recently been described (see Neser, 2008). The same principles apply in that the agent should preferably have a wide distribution, short generation time, limited host range, be damaging, and be relatively east to rear (Goeden, 1983; Harris, 1973; Schroeder and Goeden, 1986). Ease of rearing is not often considered when selecting agents (Goeden, 1983) but there are cost implications in selecting agents that are notoriously difficult to breed. On average, each agent tested and introduced requires three scientist-years (Harris, 1991). The estimated cost of research into the biological control of six invasive plant species in South Africa (in 2000 rand values) was ZAR41.1 million (van Wilgen *et al*., 2004).

The reason for considering another agent for the control of *B. delagoense* is that the more host specific and damaging agents that can be introduced to a country the higher the probability that the target weed will be controlled (Denoth *et al*., 2002; Harris, 1981; Hoffmann and Moran, 1998). The highest levels of weed biocontrol ‘success’ have been achieved using multiple agents (Baars and Heystek, 2003; DeBach, 1964; Denoth *et al*., 2002; Hoffmann and Moran, 1998). This is as a result of agents “working together” and providing a cumulative or synergistic effect (Hoffman and Moran, 1998) or because there is a greater probability that at least one of the agents that is released will be effective (Denoth *et al*., 2002). This is contrary to Myers (1985) who suggested that successful biocontrol was more frequently achieved by a single insect species than by the cumulative effect of several insect species.

Denno *et al*. (1995) also found that there were competitive interactions between two species amongst biocontrol agents in 91% of the 45 experimental studies examined. This could be as a result of direct competition for the same resource or indirect suppression of a resource by one agent required by another (Denoth *et al*., 2002). It is therefore important that agents complement each other and don't compete for the same resource, which in this case will be a particular plant part for feeding or reproduction which may result in the displacement or reduction in effectiveness of one of the introduced biocontrol agents. This said resource partitioning only dampens, but does not preclude competition between temporally and spatially separated species (Denno *et al*., 1995). It is therefore expected that competitive interactions between *O. tenuipes* and *E. bryophylli* will be reduced because *O. tenuipes* develops in the plant stem while *E. bryophylli* larvae feed and develop in the leaves. Although *O. tenuipes* adult feeding may have an impact on *E. bryophylli* larval feeding in the leaves, it is unlikely that this will have a major impact on the overall *E. bryophylli* population. In fact both species were often found in abundance at the same sites in Madagascar.

This chapter provides novel information on a leaf-mining eurytomid collected on *B. delagoense*, unknown to science before this study commenced, with information on its biology, host range and impact. We consider some of the attributes of *E. bryophylli*, to ascertain if it has potential as a biological control agent for *B. delagoense* in Australia. It follows on from the preceding chapter which looked at the potential of *O. tenuipes*, a damaging stem-boring weevil with a limited host range and rapid generation time, as a biocontrol agent. Based on preliminary host range trials *Eurytoma bryophylli* would appear to also have potential as a biocontrol agent in Australia. Although it is not as damaging as *O. tenuipes*, it has a short generation time and would be able to build-up populations very quickly, complementing damage caused by the weevil. The wide distribution of *E. bryophylli* in southern Madagascar indicates that it may be tolerant of a wide range of climatic conditions, another beneficial trait (Goeden, 1983). It leads on to the next chapter which investigates the potential of a chrysomelid beetle, *Rhembastus* sp., which feeds and develops on the roots and plantlets of *B. delagoense*, as an additional biocontrol agent to complement *O. tenuipes* and *E. bryophylli* should they ever be released in Australia.

Andrew McConnachie and Sharon Docherty were both technicians at the time and assisted with the running of some of the experiments. The paper was published in the Journal African Entomology 12(2): 201-207 (2004).

Distribution and aspects of the biology and host range of *Eurytoma* sp. (Hymenoptera: Eurytomidae), a candidate agent for the biological control of *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz (Crassulaceae) in Australia

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Surveys were conducted in Madagascar for potential biological control agents for *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz, an invasive plant in Australia. The phytophagous wasp *Eurytoma* sp. (Eurytomidae: Eurytominae) was found throughout the natural distribution of its host plant, *B. delagoense,* in southern Madagascar and was imported into South Africa for life history studies and preliminary host-range testing. The wasp proved to be a suitable candidate agent because it was easy to rear, had multiple generations per year and was damaging to its host plant in the field and under laboratory conditions although measurements would be required at the landscape scale to assess its impact on plant abundance and distribution. Eggs are deposited in the plantlets (bulbils), leaves and/or seedling stems and the larvae complete their development in the plant. There are five larval instars and pupation occurs in excavated pupal chambers within the plant. Adults emerge from plants 5–10 days after pupation and are short-lived. Preliminary host range trials revealed that *Eurytoma* sp. is oligophagous with larvae being able to complete their development on only three other species in the Crassulaceae. Extensive host range trials still have to be undertaken in Australia before the wasp can be considered for release as a potential biological control agent for *B. delagoense.*

Key words: biology, *Bryophyllum delagoense,* Crassulaceae, distribution, Eurytomidae, *Eurytoma* sp., host range, Madagascar.

INTRODUCTION

The first recorded outbreak of *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz (Crassulaceae, ‘mother-of-millions’) in Australia occurred in 1940 near Chinchilla, Queensland (Hannan-Jones & Playford 2002). The weed has subsequently become increasingly abundant in Queensland and is particularly prolific in the western Darling Downs districts and also on the northwest plains and coastal strip of New South Wales. Landholders are particularly concerned because of the plant’s toxicity to livestock (McKenzie & Armstrong 1986) and its ability to spread rapidly, either vegetatively through plantlets (bulbils) which drop off the ends of the leaves, or by seed.

In 1999, Queensland’s Department of Natural Resources and Mines initiated a biological control programme against *B. delagoense* with the establishment of a field station in South Africa. Several surveys were undertaken in the native range of *B. delagoense* in the south of Madagascar and these revealed a number of potential biological control agents, including the phytophagous wasp *Eurytoma* sp.(Hymenoptera: Eurytomidae: Eurytominae) (Witt & Rajaonarison 2004). The larvae are leafminers, causing the leaves to desiccate and drop off the plant. At high densities in the laboratory, a mature plant can lose all of its leaves. This paper reports on the distribution of *Eurytoma* sp. in southern Madagascar and aspects of its biology and host range that were studied in quarantine in South Africa.

MATERIAL AND METHODS

Field observations

Seventy-five sites with *B. delagoense* were surveyed in southern Madagascar to record the presence of *Eurytoma* sp. larvae, adults and/or damage, during four separate trips in August 1999, February and December 2000, and June 2001 (Fig. 1). For additional host records, the related crassulaceous species *Kalanchoe linearifolia* Drake and *K. beharensis* Drake growing in association with *B. delagoense* were each surveyed at more than five localities on at least two occasions. *Bryophyllum pubescens* Baker, *B. fedtschenkoi* Hamet & Perrier, *B. pinnatum* (Lam.) Persoon and *B. proliferum* (Bowie) Hamet, which were rarely found in the survey area, were only examined at one or two localities in the field, or in cultivation.

Rearing

Whole plants with larval-infested leaves were collected in Madagascar and shipped to the South African Field Station based at the ARC-Plant Protection Research Institute (ARC-PPRI) in Pretoria, South Africa in February 2000. Infested plants were potted and placed in large cages (65× 40 × 43 cm) that were maintained in a quarantine greenhouse under natural light at about 52 % relative humidity and 25 °C. All emerging adults were removed with an aspirator and placed on potted plants in adjoining cages. Voucher specimens of the wasp were lodged with the Biosystematics Division (ARC-PPRI, Pretoria, South Africa) and at the Centre de la Recherche Appliquée au Développement Rural (FOFIFA, Antananarivo, Madagascar).

Life history

Adults were randomly removed from the laboratory culture and 30 individuals were placed in each of 10 sleeves on potted *B. delagoense* plants for approximately 18 hours. Each sleeve consisted of an empty two-litre plastic bottle with the bottom end removed and gauze-covered openings for ventilation. The bottom end of the sleeve was sealed with a high-density foam disc, which had a slit cut into it to allow for the placement of the plant stem. The adults were exposed to about 16 cm of stem and approximately 50 leaves. Potted plants with sleeves were placed in a controlled environment room which had a photoperiod of 14 h L:10 h D, mean (±S.D.) temperature of 25.6 ± 1.2 °C (range = 22.9–28.3°C) and a mean (±S.D.) relative humidity of 78.9 ± 7 % (range 58.2–96.2 %). At least 10 leaves were removed from the plants every 3–4 days after adult removal and were dissected to determine the stage of larval and/or pupal development. The number of larvae, pupae and adults found in the dissected leaves were recorded and their size was measured using an ocular micrometer. The non-parametric, Kruskal-Wallis test was used to determine if there were significant differences between the lengths of males and females. The head capsule widths of the larvae were measured and analysed using cluster analysis to determine the number of instars (Payne *et al.* 1989; Anonymous 2000).

The survival and longevity of *Eurytoma* sp. adults were determined simultaneously, by comparing their survival in ventilated plastic jars, containing either a 1 % honey-water solution, a damaged *B. delagoense* seedling (exuding plant sap), or nothing. To avoid any accidental drowning of adults, the honey-water solution was placed in a small vial sealed with a wick that remained saturated for the duration of the trial. A single newly emerged adult (younger than 24 hours) was placed in each of 20 jars for each treatment. Equal numbers of males (n = 10) and females (n = 10) were tested for each treatment. The trial was undertaken in a controlled environment room with a mean (±S.D.) temperature of

25.7 ± 1.6°C (range 23.2–27.9°C) and mean (±S.D.) relative humidity of 74.6 ± 7.3 % (range 41–91.7 %). Survival was monitored every 24 hours until the last adult had died. Means for all treatments were compared by one one-way ANOVA using GenStat for Windows (Anonymous 2000).

To determine which *B. delagoense* organs were used for oviposition, leaves and plantlets (bulbils) from mature plants, and stems and leaves from seedlings, were removed from potted plants that had been exposed to *Eurytoma* sp. adults for more than 60 days. Individual leaves, stems and bulbils were placed in water in measuring cylinders to determine their approximate volume. They were then dissected and the numbers of larvae, pupae and adult emergence holes in each individual plant organ were recorded. A linear regression analysis was performed to determine if there was a relationship between the volume (size) of a plant part and the number of *Eurytoma* sp. life stages within a bulbil, leaf or stem.

Host range

A preliminary assessment of the laboratory host range of *Eurytoma* sp. was determined in no-choice trials by testing 10 species in the family Crassulaceae (including the host plant) and three species in the Asteraceae (Table 1). The three species in the Asteraceae were selected because they were morphologically similar to *B. delagoense* with succulent leaves and stems. Five males and ten females were placed in each plastic sleeve (see above) on a single potted test plant. Two plants of six of the species selected to be used in host range trials, including *B. delagoense* as a control, were tested in each trial. Plants were monitored on a daily basis for adult emergence and each trial was terminated 30 days after the first adults had emerged from the *B. delagoense* controls. Leaves and stems of all test plants were then dissected to ascertain if there was any larval development and/or damage on any of the non-target species. Trials were conducted in the quarantine laboratory under natural light at temperatures and relative humidities that ranged from 23–29 °C and 42–88 %, respectively.

RESULTS

Field observations

Evidence of *Eurytoma* sp., i.e. larvae, adults and/or damage, was found at 18 of the 75 *B. delagoense* sites surveyed in southern Madagascar (Fig. 1). *Eurytoma* sp. was particularly abundant at localities near Tsiombe in the south, Tranaroa in

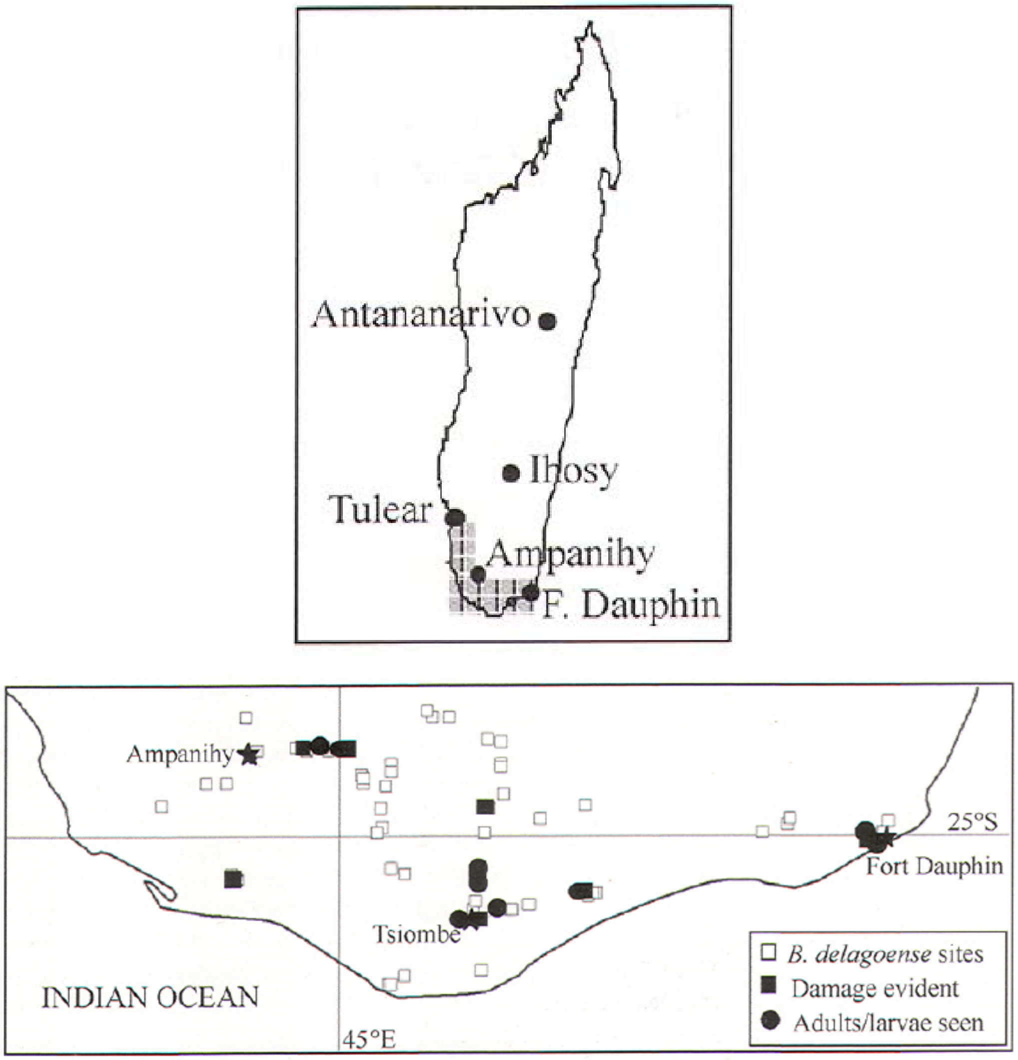

Fig. 1. Survey area in southern Madagascar (top, shaded) and the localities where *Bryophyllum delagoense* was surveyed, indicating where *Eurytoma* sp. damage was evident and where larvae and/or adults were recovered (bottom).

the north and Fort Dauphin in the east. Adults were recovered on *B. delagoense* during all four surveys, but were particularly abundant at two sites, one just west of Fort Dauphin (25°00’25”S; 46°54’48”E) in August 1999 and the other 22 km north of Tsiombe (25°07’03”S; 45°30’58”E) in February 2000. There was no evidence of *Eurytoma* sp. on any of the other species of Crassulaceae that were surveyed in Madagascar.

Life history of *Eurytoma* sp.

Eggs

Eggs are laid singly just below the epidermis of the leaf and are smooth, oblong and pale in colour. First instars were found in leaves five days after being exposed to adults indicating that the egg incubation period, is about five days or less (Fig. 2).

Larvae

Analysis of the head capsule width measurements indicated that *Eurytoma* sp. has five larval instars (Fig. 3). First instars ranged in length from 0.75–0.87 mm (n = 10) and had a mean (± S.E.) head capsule width of 0.120 ± 0.004 mm (n = 11). Final instars were 2.59±0.09mm (mean ± S.E., n = 20) long with a mean (± S.E.) head capsule width of 0.320 ± 0.001 mm (n = 20). First instars are generally pale in colour with the head capsules barely visible. Later instars are also pale-coloured but can be more easily detected in dissected leaves due to their dark internal organs. Third, fourth and fifth instars were dissected from leaves 10–19 days after adult exposure (Fig. 2).

Pupae

Pupae were initially pale-yellow with no distinctive markings but later became black with the red eyes clearly distinguishable. Pupae were found within excavated pupal chambers in leaves, plantlets or seedling stems 20 days after oviposition (Fig. 2). Pupae had a mean (± S.E.) length of 1.800 ± 0.035 mm (*n* = 20) with a mean (± S.E.) pronotal width of 0.410 ± 0.009 mm (*n* = 20).

Table 1. The laboratory host range of *Eurytoma* sp. as determined by larval survival on different test plant species during no-choice tests.

Test plants	n[2]	Adults emerged[3] (mean ± S.E.)	Days to adult emergence (mean ± S.E.)	Larval development/ feeding
Crassulaceae				
Bryophyllum delagoense[1]	5	34.40 ± 10.19	38.3 ± 0.45	Yes
B. fedtschenkoi Hamet & Perrier[1]	2	1.5 ± 0.5	33.3 ± 0.3	Yes
B. daigremontianum Hamet & Perrier[1]	2	8.5 ± 4.5	39.9 ± 1.64	Yes
B. pinnatum (Lam.) Persoon[1]	2	0	-	No
B. proliferum (Bowie) Hamet[1]	1	0	-	No
Kalanchoe blossfeldiana (von Poellnitz) Boiteau[1]	2	14.5 ± 11.5	44.1 ± 0.56	Yes
Crassula sarcocaulis Eckl. & Zeyh.	1	0	-	Yes
Crassula sp.	1	0	-	No
Echeveria sp.[1]	2	0	-	No
Asteraceae				
Senecio sp. A	2	0	-	No
Senecio sp. B	1	0	-	No
S. barbertonicus Klatt	2	0	-	No

[1] species not native to southern Africa excluding Madagascar.

[2] number of replicates.

[3] number of adults emerging from plants within 30 days after the first emergence from *B. delagoense*.

Adults

The F1 adults emerged from leaves by chewing their way out approximately 25 days after plants were exposed to the parent females (Fig. 2). Adult females (1.780 ± 0.027 mm, *n* = 13) were significantly longer than adult males (1.475 ± 0.026 mm, *n* = 10) (*H* = 15.824; *P* = 0.0001; Kruskal-Wallis test). Females have an inconspicuous petiole and a large gaster, compared to the males where the petiole is clearly visible and the gaster considerably reduced. Males and females were seen copulating and displaying oviposition behaviour, 24 hours after emergence.

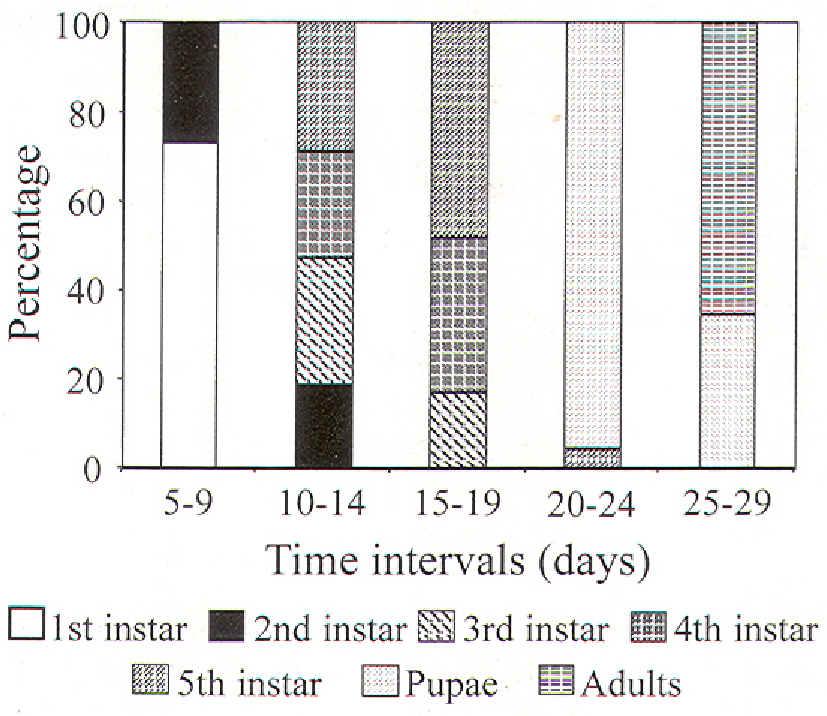

Fig. 2. Percentages of larvae and pupae of *Eurytoma* sp. found in *Bryophyllum delagoense* leaves that were dissected every 3–4 days (grouped into 5-day time intervals) after exposure to adults for approximately 18 hours. The percentage of adults that emerged is also shown.

Adult survival was extremely variable, ranging from 2–19 days. Males and females survived for longer when given access to damaged seedlings but the differences between females with access to damaged seedlings and those with access to honey-water were not significant ($P > 0.05$) (Fig. 4). There were also no significant differences in survival between males and females for each treatment.

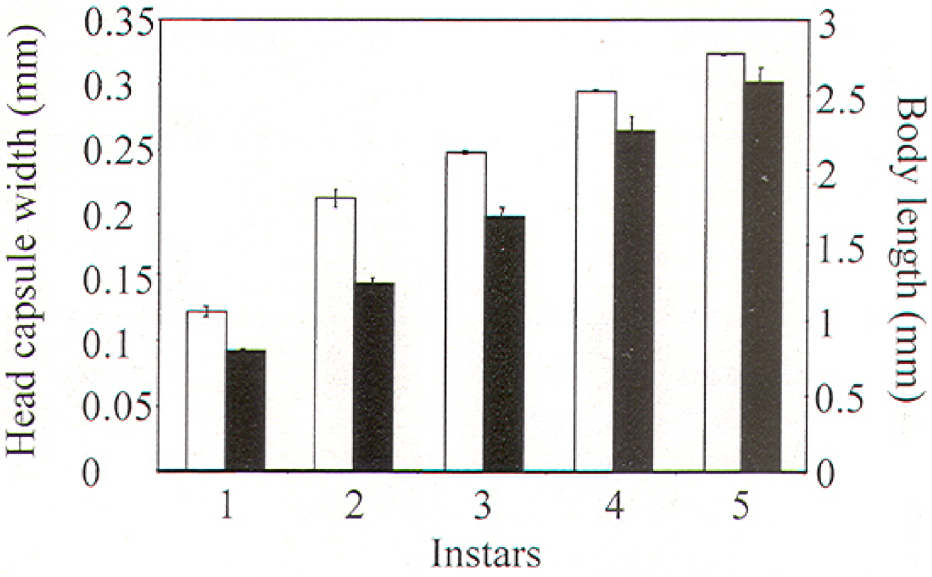

Fig. 3. Mean (± S.E.) head capsule width (open bars; y-1 axis) and body length (solid bars; y-2 axis) of the five *Eurytoma* sp. larval instars.

Oviposition sites

Eurytoma sp. larvae, pupae and emergence holes were found in mature leaves, bulbils and seedling leaves and stems. Up to 10 larvae, pupae and/or emergence holes were found in mature leaves while only 1–2 life stages were found in bulbils and seedling leaves. A regression analysis indicated that there is a strong relationship between the volume of the plant part and the number of individual *Eurytoma sp.* life stages within that organ (Fig. 5).

Host range

Preliminary host range trials indicated that *Eurytoma* sp. could complete its development on four species in the Crassulaceae, including its natural host (Table 1). Possible alternative hosts included the exotic *B. fedtschenkoi* Hamet & Perrier, *B. daigremontianum* Hamet & Perrier and *Kalanchoe blossfeldiana* (von Poellnitz) Boiteau. Although too few replicates were completed to make any definitive statements regarding host range, species that appeared to be unsuitable for development included two exotic species of *Bryophyllum,* two native species of *Crassula,* the exotic *Echeveria* sp. and three native species of *Senecio.*

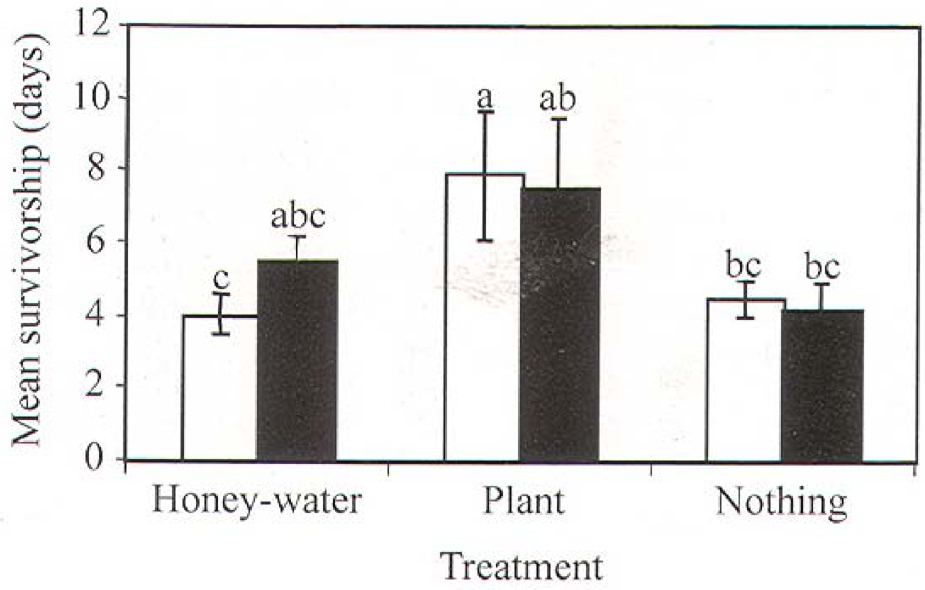

Fig. 4. Mean adult survivorship (days ± S.E.) of *Eurytoma* sp. males (open bars; n = 10) and females (solid bars; n = 10) in each of the three treatments (honey water, damaged seedlings and nothing). Bars with the same letter represent data that are not significantly different.

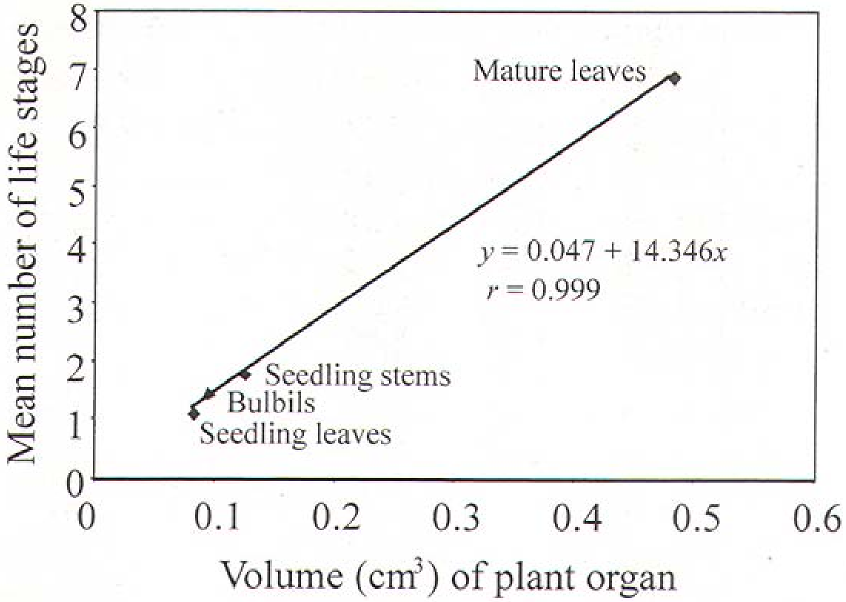

Fig. 5. Regression analysis showing the relationship between the volume of plant material and the mean number of *Eurytoma* sp. individuals (in various life stages) found in each plant part.

DISCUSSION

Based on our study, it would appear that *Eurytoma* sp. has potential as a biological control agent of *B. delagoense,* because it is easy to rear, damaging and has a suitably narrow host range. The wasp also feeds and develops on bulbils, implying that it could reduce the weed's potential for vegetative propagation. *Eurytoma* sp. also has multiple generations per year (>5 under laboratory conditions) and has a rapid developmental time of approximately 25 days which means that populations can build up relatively quickly. Further support for its potential as a biocontrol agent is evident from other studies, where congeneric species have been considered or have been utilized for the biological control of invasive plants. For example, the seed-feeding *Eurytoma attiva* (Burks) has established and is a highly effective agent for the control of *Cordia curassavica* (Jacquin) Roemer & Schultes (Boraginaceae) in Mauritius, Malaysia and Sri Lanka (Julien & Griffiths 1998). An unidentified *Eurytoma* species is being considered for the control of *Psidium cattleianum* Sabine in Hawaii (Wikler *et al.* 1996), while several *Eurytoma* species were considered for the control of *Striga* species (Bashir 1987). An unidentified *Tetramesa* species (Eurytomidae) is also currently being considered as a potential biocontrol agent for invasive *Sporobolus* spp. (Poaceae) in Australia (W.A. Palmer, pers. comm.).

In some cases, the potential of an insect to be an effective biocontrol agent can be inferred from the impact that congeneric species have as pests on cultivated crops. Crops often comprise monocultures, much the same as introduced invasive plants. In many cases crop pests are accidentally introduced in small numbers, but manage to establish readily and escape intensive predation and parasitism in their introduced range. The characteristics of many pest species may therefore render certain congeneric species attractive as biological control agents (Syrett *et al.* 1996), provided that they are monophagous or oligophagous. For example, *Eurytoma amygdali* End., a major pest of almonds, is apparently strictly monophagous in Israel (Plaut 1971), while *E. onobrychidis* Nikolskaya, a pest of sainfoin, *Onobrychis viciaefolia* Scop., in many countries including the USSR, has been reported to destroy 30–90 % of some seed crops (Richards & Hanna 1982). Furthermore, *E. onobrychidis* is apparently monophagous and less than 1 % of the insect population is parasitized in its introduced range in Canada (Richards & Hanna 1982). Other *Eurytoma* species that are pests of cultivated plants include *E. tumoris* Bugbee on *Pinus sylvestris* L. in the United States (Stark & Koehler 1964) and *E. orchidearum* (Westwood) on orchids in Hawaii and other countries (Best 1952). Based on the damage inflicted on their host plants and the narrow reported host ranges of many *Eurytoma* species, it seems likely that *Eurytoma sp.* has considerable potential as a biological control agent.

Preliminary host range trials indicated that *Eurytoma* sp. could complete its development on species other than *B. delagoense* in the family Crassulaceae. Despite these results, *Eurytoma* sp. may still have potential for the control of *B. delagoense* in Australia, as the continent has few native Crassulaceae. Indeed, there are only eight native species of Crassulaceae (all in the genus *Crassula)* in Australia (Tölken 1981) although there are several exotic crassulaceous species. None of the native species are likely to support the development of *Eurytoma* sp. because neither of the two *Crassula* species tested in this study supported its development. However, *Eurytoma* sp. completed its development on the only *Kalanchoe* species tested in these trials, namely *K. blossfeldiana.* While there is debate as to whether the related *K. crenata* (Andr.) Haw. is a native or introduced species in Australia, this species is geographically isolated, being located in the northwest of Australia, far from *B. delagoense* infestations in Queensland (W.A. Palmer, pers. comm.) Nevertheless, native Australian Crassulaceae and other beneficial plants will need to be tested before *Eurytoma sp.* can be considered for release in Australia.

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CHAPTER 6

DISTRIBUTION, BIOLOGY AND HOST RANGE OF *RHEMBASTUS SP.* (COLEOPTERA: CHRYSOMELIDAE), A CANDIDATE FOR THE BIOLOGICAL CONTROL OF *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE) IN AUSTRALIA

Justification

It is considered by some researchers beneficial to release a number of agents to control a specific weed provided that they all complement each other and do not compete for the same resource (Denoth *et al*., 2002). Releasing many agents also improves the odds that at least one of the released agents will establish and be effective (Denoth *et al*., 2002; Schroeder and Goeden, 1986). *Rhembastus* sp. does not compete directly with *O. tenuipes* or *E. bryophylli* in that its larvae develop in and on the roots of *B. delagoense* and the adults feed on the new growth, flower buds, and plantlets. By feeding on the plantlets, which grow on the apex of the leaves and which establish by dropping to the ground, *Rhembastus* sp. has a direct impact on the reproductive potential of *B. delagoense*.

Bryophyllum delagoense infestations are characterized by a mother plant surrounded by plantlets at various stages of development, similar to infestations of *B. daigremontiana* and other species in the genus. According to Herrera and Nassar (2009) *B. daigremontiana* plantlets of asexual origin show high survival (75-100%) compared to seedlings of sexual origin (10%); *B. daigremontiana* is autogamous with a high seed ouput of more than 16,000 seeds per fruit. Herrera *et al.* (2011) concluded that seedling recruitment contributed little to the transient dynamics of *B. daigremontiana* populations and therefore could not warrant the successful establishment of the species. We therefore assume the same or similar for *B. delagoense*. Although adult feeding of flower buds by *Rhembastus* sp. reduces seed production it is the feeding on the plantlets which has a bigger impact on the reproductive potential of *B. delegoense*. *Rhembastus* sp. is therefore the only potential agent that has a direct impact on *B. delagoense* reproduction. No seed-feeding insects were found in Madagascar (Witt and Rajaonarison, 2004). Another important consideration in selecting *Rhembastus* sp. as a prospective agent is that chrysomelids have immense potential as biological control agents and many species have been used in the control of weeds throughout the world (Crawley, 1989; Julien and Griffiths 1998; Syrett *et al*., 1996).

This chapter contributes to our existing knowledge on classical biological control by revealing the biology, host range and impact of a potential biological control agent for *B. delagoense*, a species which was unknown to science prior to the commencement of this study. This follows-on from the preceding chapters where we evaluated the potential of *O. tenuipes* and *E. bryophylli* as potential biocontrol agents. We also provide some background information with regard to the taxonomy of *Rhembastus* sp. because Madagascan chrysomelids have been poorly studied. This information is often critical in ascertaining if a particular species, selected as a potential biological control agent, or any of its closely related congeners, have been recorded as pests of any agricultural crops anywhere in the world e.g. *Leptinotarsa texana* Schaeffer (Chrysomelidae) on *Solanum eleagnifolium* Cavanilles (Solonaceae) vs *L. decemlineata* Say on *S. tuberosum* L. (see Olckers *et al*., 1999). A potential agent which is a known crop pest can then be rejected at the initiation of a biocontrol programme before commencing with expensive host-range trials (Schroeder and Goeden, 1986). Species in the genus *Rhembastus* have been found on a variety of plant species and recently a species was collected on *Macadamia integrifolia* Maiden & Betche (Proteaceae, macadamia nuts) near Nelspruit, South Africa (van den Berg, 2000). This may be cause for concern since Australia has a large macadamia nut industry but host-range tests found that the *Rhembastus* species collected on *B. delagoense* has a sufficiently narrow host range to be considered for further testing as a potential biocontrol agent in Australia – *M. integrifolia* should be included in a test list.

Adults and/or feeding damage by *Rhembastus* sp. was evident at 40 of the 75 known *B. delagoense* sites surveyed across the whole of southern Madagascar, from Ampanihy in the west to Fort Dauphin in the east. The average annual temperature in Ampanihy is 24.7°C per annum with an average annual rainfall of 581 mm, compared to 1588 mm in Fort Dauphin, which has more of a tropical climate with an average annual temperature of 22.9°C per year. Rain falls throughout the year in Fort Dauphin, but is largely unimodal in Ampanihy. This is an indication that *Rhembastus* sp. may be tolerant of a wide range of climatic conditions, and therefore more likely to establish if released in Australia. If released, it will join other root-feeding chrysomelids such as *Longitarsus flavicornis* (Stephens), *L. jacobeae* (Waterhouse), *L. aeneus* Kutschera, *L. echii* Koch and *L. albineus* (Foudras) (Julien and Griffiths, 1998), all of which have been released in Australia for the control of various invasive plant species. This particular chapter leads onto the next, which attempts to ascertain if newly acquired natural enemies on *B. delagoense* in southern Africa are preventing *B. delagoense* from reaching pest status.

Andrew McConnachie was a technician at the time of the study and assisted with many of the experiments, while Beth Grobbelaar was a taxonomist at the National Collection of Insects (ARC-PPRI) who provided valuable insights into the identification and taxonomy of *Rhembastus* sp. Bill Palmer was my employment supervisor and provided guidance during the study. The paper was published in BioControl, Science and Technology 16(8): 859-869 (2006).

Distribution, biology and host range of *Rhembastus sp.* (Coleoptera: Chrysomelidae), a candidate for the biological control of *Bryophyllum delagoense* (Crassulaceae) in Australia

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Abstract

The biology and host range of a leaf-beetle, *Rhembastus* sp. (Coleoptera: Chrysomelidae: Eumolpinae) from Madagascar, was studied under quarantine laboratory conditions in South Africa to determine its potential as a biological control agent of *Bryophyllum delagoense* (Ecklon & Zeyher) Schinz (Crassulaceae) in Australia. Favourable attributes of the beetle include relative ease of culturing, multiple generations per year, and high levels of damage inflicted by adults, which feed on the plantlets produced at the end of each leaf, and root feeding larvae. The adults therefore have an impact on the reproductive potential of the plant and larval feeding on the roots hampers the uptake of water and may even facilitate secondary infections by pathogens. Despite indications from field surveys in Madagascar that *Rhembastus sp.* has a narrow host range, preliminary no-choice and multiple-choice trials in quarantine revealed that it could complete its development on five non-target species in the family Crassulaceae. Extensive host range trials still have to be undertaken in Australia before the beetle can be considered for release.

Keywords: *Bryophyllum* delagoense, Chrysomelidae, host range, Madagascar, *Rhembastus* sp., weed biocontrol

Introduction

Bryophyllum delagoense (Eckl. & Zeyh.) Schinz (Crassulaceae, 'mother-of-millions'), an introduced plant from Madagascar, was first recorded in Australia in the 1940s (Hannan-Jones & Playford 2002). Since its introduction into Australia, it has invaded thousands of hectares of grazing land, particularly on the Darling Downs and in central Queensland where it is spreading rapidly down river drainage systems with each flood event (Hannan-Jones & Playford 2002). In central Queensland, it threatens the survival of the endangered bridled nail-tailed wallaby, *Onychogalea fraenata* Gould, by competing with native herbs and forbs that are the diet of this macropod (Hannan-Jones & Playford 2002). The flowers of *B. delagoense* are also especially poisonous to livestock having caused a number of cattle deaths (McKenzie & Armstrong 1986) with the single most extensive stock loss of 122 head of cattle recorded in the Australian State of New South Wales (Hannan-Jones & Playford 2002). The leaves are grey-brown and fleshy with up to seven projections at the tip of each leaf, which produce new plantlets (bulbils), which drop to the ground and form new plants. Each flower head produces thousands of seeds, with 57% germinating in laboratory trials after 38 days (Hannan-Jones & Playford 2002). Mechanical and chemical control of heavy infestations is expensive and often ineffective, and because this plant has few natural enemies in Australia (Hannan-Jones & Playford 2002), surveys for potential biological control agents were undertaken in Madagascar (Witt & Rajaonarison 2004).

A number of promising insect species were collected on *B. delagoense* during surveys in Madagascar (Witt & Rajaonarison 2004). A chrysomelid beetle, *Rhembastus* sp. was abundant and found to be very damaging at a number of sites. The genus *Rhembastus* Harold, 1877 is known from the Ethiopian Region (Seeno & Wilcox 1982) and by 1914 some 18 species had already been recorded from Madagascar (Clavareau 1914). Originally regarded to be a very large genus, Selman (1965) reduced the size of the genus considerably when he transferred 45 species to other genera leaving 35 species. He indicated that it was rare to find the same chrysomelid genus occurring in Africa and Madagascar and it was for this reason that he had not worked on the latter fauna in 1965. He stated that the Madagascan species currently placed in the genus *Rhembastus* did not possess the distinctive characteristics for this genus. This was confirmed by E. Grobbelaar when only two of the four character states used by Selman (1965) to define the genus *Rhembastus* were found on *Rhembastus* sp. Selman (1965) purposefully left the Madagascan '*Rhembastus*' species, and those in two other genera, in the genera in which they were described until the Eumolpinae of Madagascar would one day be revised. He suggested that this would produce a complete generic separation of the African and Madagascan species. According to Dr R. Wills Flowers (Center for Biological Control (FAMU-CESTA), Florida A&M University, pers. comm.), a specialist on Eumolpinae, a full-scale revision of the Madagascan fauna is required before *Rhembastus* sp. can be formally identified. This will entail studying specimens collected by Bechyné, who described many Madagascan eumolpine species (see Bechyné 1950, 1953, 1956) all without keys and illustrations. Dr M.L. Cox (Entomology Department, Natural History Museum, London, pers. comm.) determined that *Rhembastus* sp. compared well with specimens in the *Rhembastus* (*antennatus* Jacoby) group. As *Rhembastus* antennatus Jacoby 1892, is a Madagascan species, the *Rhembastus* sp. specimens collected on *B. delagoense* can be well placed, despite the fact that they cannot be identified to species level.

The uncertain taxonomic status of *Rhembastus* sp. should not detract from its potential as a biocontrol agent as it causes considerable damage to *B. delagoense* plants. Adults feed ectophagously on new growth, bulbils and occasionally on mature leaves, while larvae are root-feeders. *Rhembastus* sp. will also compliment two other candidate biocontrol agents, a stem-boring weevil and a leaf-mining eurytomid (Witt & Rajaonarison 2004). Chrysomelids have immense potential as biological control agents and many species have been used in the control of weeds throughout the world (Syrett et al. 1996; Julien & Griffiths 1998). These factors supported our decision to assess the potential of *Rhembastus* sp. as a biological control agent. This paper examines the biology and host range of this beetle in South Africa prior to shipment for further testing in Australia.

Materials and methods

Field observations

The distribution of *Rhembastus* sp. in southern Madagascar was ascertained by examining different species of Crassulaceae in the field for the presence of adults and/or feeding damage. *Kalanchoe beharensis* Drake and *K. linearifolia* Drake growing in association with *B. delagoense* were examined at six and 11 sites, respectively. *Bryophyllum* pubescens Baker, *B. fedtschenkoi* Hamet & Perrier, *B. pinnatum* (Lamarck) Persoon, *B. proliferum* (Bowie) Hamet and *B. daigremontianum* Hamet & Perrier were rarely found in the field and only examined at two or three sites (Table I). None of the congeners examined were found growing in association with *B. delagoense* in the field.

An average of approximately 70 *B. delagoense* plants were surveyed at each site for arthropods and pathogens during four separate trips to Madagascar in August 1999, February and December 2000 and in June 2001 (Witt and Rajaonarison 2004). The phenology of *Rhembastus* sp. was determined by recording the presence of adults on plants.

Biology

Eighty-six adults feeding on new growth and bulbils were collected, using an aspirator, in Tulear Province, southern Madagascar in February 2000, and imported into South Africa. Adults were placed in large cages (650 x 400 x 430 mm) on potted *B. delagoense* plants in quarantine laboratories of the ARC-Plant Protection Research Institute (ARC-PPRI) in Pretoria, South Africa. All plants used in trials were collected from the field in and around Pretoria and planted in a well-drained soil mixture (sand: compost, 1:2) in 2-L pots. Plants were maintained in the quarantine glasshouse under natural light and replaced every 10-14 days. Exposed plants were watered at regular intervals.

Voucher specimens of the beetle from the laboratory reared culture have been lodged with the National Collection of Insects (SANCI, ARC-PPRI, Pretoria, South Africa), Natural History Museum, London, United Kingdom (BMNH) and at the Centre National de la Recherche Appliquée au Développement Rural (FOFIFA), Antananarivo, Madagascar.

Table I. Plant species in the Crassulaceae surveyed in Madagascar for the presence of *Rhembastus* sp.

Species	Localities surveyed	Occasions[a]	*Rhembastus* sp. present[b]
Bryophyllum delagoense	75	1-4	40
B. pubescens	2	1	0
B. pinnatum	2	1	0
B. proliferum	2	1	0
B. daigremontianum	3	1	0
B. fedtschenkoi	2	1	0
Kalanchoe linearifolia	6	2	0
K. beharensis	11	2	0

[a] Number of occasions each locality was surveyed.

[b] Number of localities *Rhembastus* sp. damage recorded and/or adults present.

Development of *Rhembastus* sp. was determined by placing 50 adults in each of 10 sleeves, with each sleeve placed on a randomly selected potted *B. delagoense* plant, for 72 h. Each sleeve consisted of an empty 2-L plastic bottle, approximately 160 mm in height, with the bottom end removed and gauze-covered openings for ventilation. The bottom end of the sleeve was pushed into the soil allowing adults access to the soil. Soil and roots of each of the ten plants were removed at intervals of 3-11 days (sampling interval dependant upon the detection of new life stages) after having been exposed to adults, and examined under a dissecting microscope. The number and size of eggs, larvae and pupae was recorded using an ocular micrometer fitted to an Olympus SZX9 microscope. The data on larval head capsule widths were analysed using cluster analysis (Payne et al. 1989). Non-hierarchial clustering was used to determine the number of instars. The criterion used for determining the optimum classification was the minimum within-group sum of square’s method. After the optimum classification was obtained an analysis of variance was used to test for differences between the instars. Instar means were separated using the Fishers' protected t -test (Snedecor & Cochran 1980). All development trials were undertaken in a constant environment room, which had a photoperiod of 14 h L:10 h D and mean (±SD) temperature of 25.7±1.6°C (range = 22.9-29.9°C) and mean (±SD) relative humidity of 59.5±7.9% (range 38-85.3%).

Adult longevity was determined by placing one recently emerged (within 24 h) adult, in each of 10 well-ventilated plastic containers, on *B. delagoense* cuttings. Cuttings were replaced every 7 days. Containers were placed in a constant environment room, under the same conditions as for the above trials.

Preliminary laboratory host range trials

No-choice adult feeding trials. Trials were undertaken to determine the laboratory host range of *Rhembastus* sp. Adults were exposed to 12 species in the Crassulaceae and one each in the Portulacaceae and Asteraceae (Table II). These species were tested because they are taxonomically related to *B. delagoense* or are similar morphologically with succulent leaves or stems. In each trial 10 unsexed adults were placed in each of six cages (650 x 400 x 430 mm). Five cages each had a single test plant and one cage contained *B. delagoense* as a control. Due to a limited number of test plants available each plant species was only tested once or twice. After 10 days, all adults were removed and the number of live adults recorded. Feeding damage was categorized on an absence/presence basis.

No-choice adult feeding and larval development trials. In additional no-choice trials three plants of each of six species (Table III) were exposed to 30 adults for 10 days, after which the adults were removed. The number of live adults was recorded and feeding damage was categorized on an absence/presence basis. The three plants of each individual species, used in each trial, were then retained in a single cage and monitored on a weekly basis for 1 year. The number of emerging adults was recorded. All trials were conducted in the quarantine glasshouse under natural light at temperatures ranging from 21-26°C and relative humidity from 40-90%.

Multiple-choice adult feeding and larval development trials. In no-choice trials potential agents often exhibit a wider host range than would normally occur in the field. Multiple-choice trials are often undertaken to determine if these results are due to aberrant host selection behaviour (Heard 1997). Five of the species used in no-choice trials; three that had, and two that had no *Rhembastus* sp. feeding damage and *Kalanchoe blossfeldiana* were selected for use in this trial. In each cage (650 x 400 x 430 mm), single plants of three of the non-target species and *B. delagoense* were exposed to 30 *Rhembastus* sp. adults, which were removed after 10 days. Two plants of each of the six non-target species were tested in this way. Feeding damage was then categorized as absent, negligible (>25%), moderate (25-50%) or substantial (<50%). Roots and soil of all of the six non-target species and *B. delagoense* were removed and examined under a dissecting microscope 6 weeks after adult exposure. All larvae and pupae in the soil were counted.

Results

Field observations

Rhembastus sp. adults were observed, or damage was recorded, at 40 of the 75 sites where *B. delagoense* was surveyed in southern Madagascar (Figure 1). *Rhembastus* sp. was not found on any other species examined in Madagascar including a number of *Kalanchoe* and other *Bryophyllum* spp. (Table I).

Based on the surveys which covered most of the known distribution of *B. delagoense*, the beetle has a wide distribution from near Anavaho (25^{o}09’46”S; 44^{o}38’09”E) in the west, to north of Fort Dauphin (24^{o}57’07”S; 46^{o}59’23”E) in the east, and near Bikitro (24^{o}33’26”S; 45^{o}19’30”E) in the north, to north of Faux Cap (25^{o}29’33”S; 45^{o}31’03”E) in the south (Figure 1). *Rhembastus* sp. adults were seen at 25 of the 75 sites surveyed (Witt & Rajaonarison 2004). They were seen feeding on new growth and bulbils during surveys in December and February, while a few adults were found feeding on flower buds and petals in June. This would suggest that *Rhembastus* sp. has multiple generations per year in the field. However, no adults were seen in surveys during August, which suggests that *Rhembastus* sp. enters diapause during the colder and drier periods in southern Madagascar.

Table II. Laboratory host range of *Rhembastus* sp. as determined by adult survival on different test species during no-choice trials.

Test plants	*N*	Adults alive after 10 days (mean ± SE)	Feeding damage
Crassulaceae			
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz[a]	5	8.4 ± 0.5	Yes
B. pinnatum (Lam.) Persoon[a]	2	9 ± 0	Yes
B. proliferum (Bowie) Hamet[a]	2	8.5 ± 0.5	Yes
B. daigremontianum Hamet & Perrier[a]	2	6.5 ± 0.5	Yes
B. fedtschenkoi Hamet & Perrier[a]	1	8	Yes
Kalanchoe beharensis Drake[a]	1	2	No
K. orgyalis Baker[a]	1	1	No
K. sexangularis N.E.Br[b]	1	9	Yes
Crassula ovata (Mill.) Druce[b]	1	3	Yes
C. sarmentosa Harv.[b]	1	1	No
Sedum sp.	1	5	Yes
Echeveria sp.	2	6.5 ± 0.5	Yes
Portulacaceae			
Portulacaria afra Jacq.[b]	2	0.5 ± 0.5	No
Asteraceae			
Senecio barbertonicus[b]	2	0 ± 0.0	No

[a]Species native to Madagascar.

[b]Species native to southern Africa excluding Madagascar.

Biology of Rhembastus *sp.*

Rhembastus sp. adults had a mean (± SE) body length of 3.64 ± 0.07 mm (range 2.82-3.98 mm) (*n* = 25). The body is elongate-ovate with the prothorax as wide as the elytra basally but narrowing towards the head in dorsal view. The head, thorax and elytra are reddish-brown with the antennal segments seven and nine to eleven mostly brownish- black. The head retracts into a hood-like prothorax, covering the posterior margin of the eyes, which are large, ovate and deeply emarginate around the antennal sockets. The antennae are filiform and short, less than half the body length, with the basal segment globose and the second segment distinctly shorter than the third and convexly rounded dorsally. The thorax width at the basal margin was 1.4x ± 0.00 (± SE) (range = 1.28-1.68 mm) (n = 25) wider than the width at the anterior margin and covered with small distinct punctures. The legs are short and the femora are armed with a ventral spine. The mid- and hind tibiae are emarginated and the claws bifid. The elytra are at least 2.39x ± 0.01 (± SE) (range = 2.25-2.49 mm) (*n* = 25) the medial length of the pronotum and punctate-striate with the intervals flat. We found no easily discernable, external differences between males and females.

Eggs were white in color and had a mean (± SE) length of 0.49 ± 0.01 mm and a mean (± SE) width of 0.25 ± 0.01 mm (*n* = 20). Eggs were found in the top 1-2 cm of soil, 14 days after potted plants had been exposed to adults for 72 h (Figure 2).

Larvae were found feeding internally and externally on the roots. They are elongate and more or less C-shaped (scarabaeiform), with the dorsal and lateral surfaces very lightly sclerotized and sparsely setose. The first segment is slightly more heavily sclerotized and lightly pigmented dorsally with the head well sclerotized, yellowish-brown and slightly retracted. The legs are well developed and five-segmented, each with a long, curved, unisetate tarsungulus. Analyses of the head capsule width measurements suggest that *Rhembastus* sp. has six larval instars. First instar larvae, some of which were found in the soil 14 days after being exposed to adults, have a mean (±SE) head capsule width of 0.20 ± 0.00 mm (*n* = 20). Final instar larvae have a mean (±SE) head capsule width of 0.73 ± 0.01 mm (*n* = 30) and were first found 58 days after plants were exposed to ovipositing adults. In these trials the final instar appeared to enter diapause which meant that the duration of the pupal stage and the time taken for *Rhembastus* sp. to complete its development could not be accurately determined. However, casual observations during mass rearing, in mid-summer, seem to suggest that the beetle takes approximately 75 days to develop from egg to adult which would indicate, based on the results of the above-mentioned trial, that the final instar and pupal stage combined is in the region of 17 days or even less.

Table III. Number of adults emerging within a year from three plants of each species exposed to 30 adults for 10 days during no-choice trials.

Test plants	Feeding damage	Total adults emerged
Crassulaceae		
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz[a]	Yes	18
B. pinnatum (Lam.) Persoon[a]	Yes	9
B. proliferum (Bowie) Hamet[a]	Yes	11
Echeveria sp.	Yes	14
Kalanchoe sexangularis N.E.Br[b]	Yes	38
Portulacaceae		
Portulacaria afra Jacq.[b]	No	0

[a] Species native to Madagascar.

[b] Species native to southern Africa excluding Madagascar.

Larval diapause was also observed during mass rearing with very few adults emerging during winter as opposed to summer when the plants were actively growing and producing bulbils. This is supported by data from Madagascar where no adults were collected or seen during surveys in August 1999 and only a few were collected in June 2001. Adults collected in summer were actively feeding on the terminal growth and bulbils while the few that were active in winter were feeding on flower buds and petals.

Of the 10 *Rhembastus* sp. adults used in longevity trials, two were still alive at the termination of the trial after 251 and 256 days. The other eight adults died of perceived natural causes after (mean ± SE) 155 ± 14 (n = 8) (range = 82-194) days.

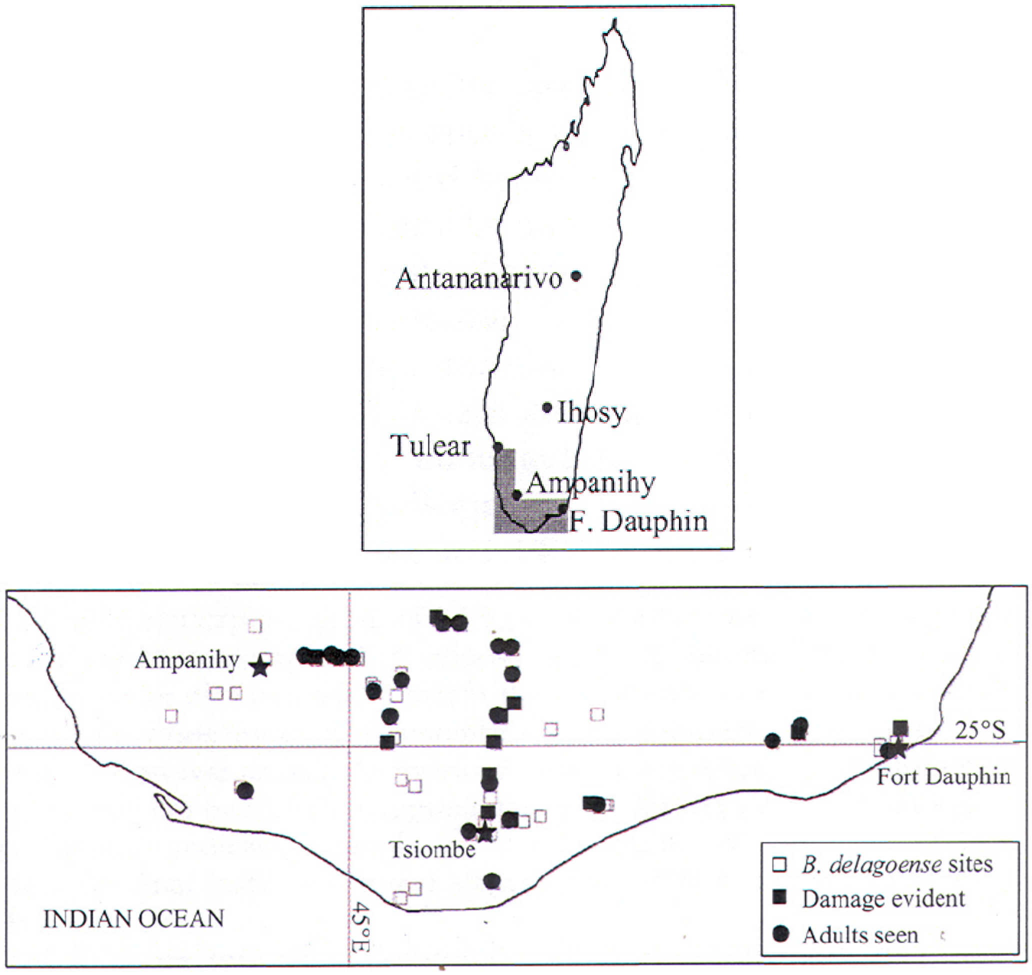

Figure 1. Survey area in southern Madagascar (top, shaded) and the localities where *Bryophyllum delagoense* was surveyed in southern Madagascar, indicating where *Rhembastus* sp. adult feeding damage was evident and were adults were recovered (bottom).

Host specificity of Rhembastus *sp*.

No-choice trials. Of the 13 non-target species tested in no-choice feeding trials *Rhembastus* sp. fed on eight species (all in the family Crassulaceae) (Table II). Survival on species with feeding damage was relatively high with the exception of *Crassula ovata* (Mill.) Druce where only three adults were still alive after 10 days. In no-choice trials, where development was monitored, *Rhembastus* sp.

could complete its life-cycle on four of the five non-target species tested (Table III).

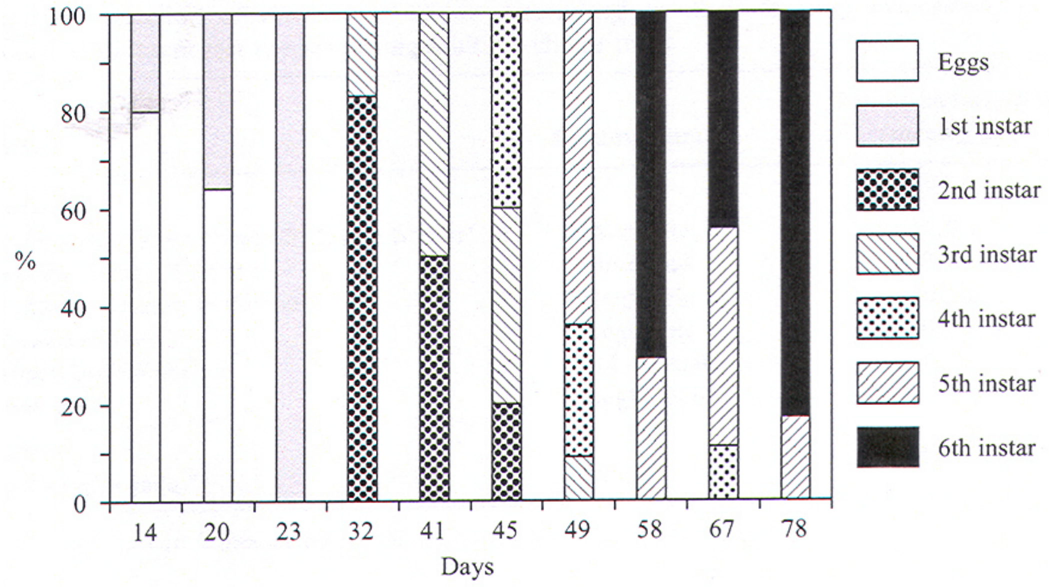

Figure 2. Percentages of eggs and larval instars removed from the soil on various days after exposure to 50 adults for 72 h.

Multiple-choice trials. Larvae were found in the soil of every *B. delagoense* plant used in each replicate (range = 7-36) and two of the six non-target species tested, both *Bryophyllum* species (Table IV). Although substantial feeding occurred on both *Kalanchoe blossfeldiana* (von Poellnitz) Boiteau plants tested, no larvae were found in the soil. Despite the fact that feeding damage on *B. fedtschenkoi* was negligible, larvae were found feeding on the roots in contrast to *Crassula sarmentosa* Harv. and *Echeveria* sp. where there were no larvae.

Discussion

Approximately 38 genera and 175 species of Eupolminae have been described from southern Africa (Scholtz & Holm 1985). Very little is known about the biology of these species especially those in the genus *Rhembastus*. According to Jolivet and Hawkeswood (1995) *Rhembastus* species have been collected on a

Table IV. Host selection of *Rhembastus* sp. as determined by their feeding, oviposition and larval development on different test species during multiple-choice trials.

Test plants	*n*	Feeding damage	No. of larvae/pupae (mean ± SE)
Crassulaceae			
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz[a]	4	Moderate	21.5 ± 4
B. pinnatum (Lam.) Persoon[a]	2	Moderate	8 ± 3
B. fedtschenkoi Hamet & Perrier[a]	2	Negligible	16 ± 4
C. sarmentosa Harv.[b]	2	Negligible	0
Kalanchoe blossfeldiana[a]	2	Substantial	0
Echeveria sp.	2	Negligible	0
Portulacaceae			
Portulacaria afra Jacq.[b]	2	None	0

[a]Species to Madagascar.

[b]Species native to southern Africa excluding Madagascar.

wide range of plants in different families including *Acacia* (Mimosaceae), *Tephrosia* (Fabaceae), *Helianthus* (Asteraceae), *Coffea* (Rubiaceae), *Malus* (Rosaceae), *Gossypium* (Malvaceae) and *Hibiscus* (Malvaceae). Recently a *Rhembastus* sp. was also collected on *Macadamia integrifolia* Maiden & Betche (Proteaceae) near Nelspruit, South Africa (van den Berg 2000). According to Jolivet and Hawkeswood (1995) only three species of chrysomelids have ever been collected on species in the Crassulaceae and only one of those, *Geinella* sp. (Galerucinae) is thought to be able to complete its development on a species of *Rhodiola*. Surveys in Madagascar on *B. pinnatum* during this study resulted in the discovery of an *Aphthona* sp., which is a new record of a chrysomelid on a species in the Crassulaceae (A.B.R. Witt, pers. obs.).

Chrysomelids from the subfamily Alticinae, with root-feeding larvae, have been utilized as biological control agents for a number of invasive plant species.

This is an indication that some root-feeding chrysomelids do have restricted host ranges. *Longitarsus flavicornis* (Stephens) and *L. jacobeae* (Waterhouse) have been released for the control of *Senecio jacobeae* L. (Asteraceae) in Australia and elsewhere (Julien & Griffiths 1998). *Longitarsus aeneus* Kutschera and *L. echii* Koch were released in Australia for the control of *Echium plantagineum* L. (Boraginaceae), while *L. albineus* (Foudras) was released for the control of *Heliotropium europaeum* L. (Boraginaceae), also in Australia (Julien & Griffiths 1998).

Preliminary host range trials indicated that *Rhembastus* sp. could complete its development on species other than *B. delagoense* in the family Crassulaceae. Despite these results *Rhembastus* sp. may still have potential as a biocontrol agent for *B. delagoense*, as there are only eight native species of Crassulaceae (all in the genus Crassula) in Australia (Tölken 1981) although there are several exotic crassulaceous species. In this paper *Kalanchoe crenata* (Andrews) Haw., which has been recorded in Western Australia, is regarded as introduced (see Kenneally 1983). The laboratory host range appears to be very similar to that recorded for a stem-boring weevil, *Osphilia tenuipes* (Fairmaire) (Coleoptera: Curculionidae) (Witt 2004), and a leaf-miner, *E. bryophylli* (Hymenoptera: Eurytomidae) (Witt et al. 2004), two other potential agents collected on *B. delagoense* in Madagascar. *Osphilia tenuipes* has undergone extensive host range trials in Australia and could not complete its development on any of the native Australian Crassulaceae tested (W.A. Palmer unpubl.). However, unlike *O. tenuipes* and *E. bryophylli* it would appear from the results of the multiple-choice trials that *Rhembastus* sp. cannot develop on *K. blossfeldiana*, an important ornamental plant in Australia. This is despite the fact that 30 *Rhembastus* sp. larvae were recovered from the *B. delagoense* plant used in the same replicate together with *K. blossfeldiana*. This result was unexpected considering that development occurred on a congener, *K. sexangularis* , in no-choice trials and *O. tenuipes* developed on *K. sexangularis* and *K. blossfeldiana* in multiple-choice trials (Witt 2004). However, *O. tenuipes* did not develop on all *Kalanchoe* spp. tested (Witt 2004) and *E. bryophylli* even failed to develop on *B. pinnatum* and *B. proliferum* in no-choice trials (Witt et al.

2004). Nevertheless, the results of these preliminary host range trials will have to be confirmed by more intensive studies, which will have to be undertaken in Australia, on native Crassulaceae species and other beneficial plants. If *Rhembastus* sp. is found to be suitably host-specific, it will be an excellent candidate for biocontrol because of its potential impact on the reproduction, growth and survival of *B. delagoense*, by adults feeding on the bulbils and larvae on the roots.

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Thanks to Mme Y. Rabenantoandro (FOFIFA) for providing collecting and export permits and to Mr J. Rajaonarison (FOFIFA) for assistance in the field in Madagascar. Thanks to Dr M.L Cox from the British Natural History Museum for identifying *Rhembastus* sp. to genus level and Professor Wills Flowers (Center for Biological Control (FAMU-CESTA), Florida A&M University) for his opinions concerning the Eumolpinae. Thanks also to Drs T. Olckers (UKZN) and M. Byrne (Wits) for valuable comments on previous drafts of the manuscript. Thanks also to Ms L. Morey (ARC-Biometry Unit) for assistance with the statistical analyses and to the Weeds Research Division (ARC-PPRI) for making their facilities available.

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CHAPTER 7

IS BIOTIC RESISTANCE PREVENTING *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE) FROM BECOMING INVASIVE IN SOUTHERN AFRICA?

Justification

This chapter follows-on from the preceding chapters which considered the insects associated with *B. delagoense* in its natural environment and examined the host ranges of three species. The host range tests revealed that these insects have the ability to develop on closely related species in the genus *Kalanchoe*, many species of which are native to southern Africa. This provided an impetus to examine the natural enemies associated with *B. delagoense* in southern Africa because as an introduced species it should, in theory at least, have acquired natural enemies from native *Kalanchoe* species.

According to the “Enemy Release Hypothesis” introduced species become invasive because of the absence of natural enemies in their introduced range (Keane and Crawley, 2002). However, many introduced species never become invasive due to the presence of multiple barriers including losses caused by generalist or pre-adapted natural enemies (Colautti *et al.*, 2004; Crawley, 1987; Cronk and Fuller, 1995; Richardson *et al.*, 2000) or by the absence of mutualists such as pollinators. Introduced species are more likely to acquire natural enemies from closely related plant species in the country of introduction (Colautti *et al.*, 2004; Hokkanen and Pimentel, 1989; Strauss *et al.*, 2006). This is based on the theory that closely related plants are biochemically and structurally more similar than less closely related ones (Carter and Feeny, 1999; Futuyma, 1976).

There are about 225 species in the family Crassulaceae in southern Africa (Tölken, 1985), which are hosts to a multitude of natural enemies that may be pre-adapted to feed and develop on the introduced *B. delagoense* (Eggli *et al.*, 1995).

In contrast, Australia has only eight indigenous species of Crassulaceae, all belonging to the genus *Crassula* (Tölken, 1981). In this paper we consider *K. crenata* to be introduced (see Kenneally, 1983). The probability of the introduced *B. delagoense* acquiring new natural enemies in southern Africa is therefore greater than in Australia – these newly acquired natural enemies may be reducing the fecundity and growth of *B. delagoense* in Africa and as a result limit its spread. Lack of pre-adapted natural enemies may be one of the reasons why *B. delagoense* is so invasive in Australia.

The "Evolution of Increased Competitive Ability Hypothesis" (EICA) (Blossey and Nötzold, 1995) argues that exotics, long liberated from their natural enemies should lose the costly traits that confer resistance to their native natural enemies. Exotics can reallocate resources from the maintenance of resistance to traits, such as size and fecundity. Liberation from natural enemies may result in exotics evolving in ways that enhance their performance in recipient communities. If this hypothesis is correct then *B. delagoense* is likely to be invasive in Australia because it has only acquired two natural enemies. Surveys in Australia have only found two species of insects, *Scirtothrips aurantii* and *Aphis sedi*, both of them introduced, which can complete their development on *B. delagoense* (MA Hannan-Jones, Queensland Department of Primary Industries & Forestry, pers. comm.).

This chapter provides data to support the contention that introduced plant species acquire pre-adapted natural enemies if there are closely related native congeners in the country of introduction. These novel findings are supported by evidence from the United States and Australia where the extent of the *B. delagoense* invasions were correlated with the phylogenetic relatedness to crassulaceous species in each region. We conclude that pre-adapted or generalist natural enemies may be preventing *B. delagoense* from becoming invasive in southern Africa and that *B. delagoense* does not require the services of specialized pollinators. This leads onto the next chapter where we investigate the potential of

Alcidodes sedi, a natural enemy of *B. delagoense* in southern Africa, as a potential biocontrol agent of *B. delagoense* in Australia.

Andrew McConnachie was the technician who assisted with surveys while Riaan Stals from the National Collection of Insects (ARC-PPRI) provided information with regard to the insects associated with crassulaceous species. This paper has been submitted to the journal "Austral Ecology".

Is biotic resistance preventing *Bryophyllum delagoense* (Crassulaceae) from becoming invasive in southern Africa?

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Abstract: As a result of novel biotic interactions or unsuitable abiotic conditions in the country of introduction, most introduced plant species fail to become invasive. Here we question if biotic resistance in the form of interactions with new generalists or pre-adapted natural enemies, or a lack of suitable mutualists, may be preventing *Bryophyllum delagoense* (Eckl. & Zeyh.) Schinz, a Madagascan endemic, from becoming as invasive in its introduced range in southern Africa as it has in Australia. Surveys in southern Africa revealed that 14 native and introduced insects have the ability to feed and develop on *B. delagoense*, compared to only two insect species in Australia. This can largely be explained by phylogenetic studies which indicate that introduced *Bryophyllum* species are more closely related to native Crassulaceae species in southern Africa than they are in Australia, which is an indication that the pool of potential pre-adapted natural enemies is far larger in Africa. We conclude that interactions with newly acquired natural enemies may be a contributing factor inhibiting the spread of *B. delagoense* in southern Africa, compared to Australia, after germination trials indicated that seeds collected in the former were just as viable as those in Australia. This study largely supports the contention that introduced plants more closely related to those in their new environment are more likely to acquire new natural enemies and as a result less likely to become invasive. In many respects it is a test of the "Enemy Release Hypothesis".

Key words: biotic resistance • *Bryophyllum delagoense* • mutualisms • natural enemies • pollination.

INTRODUCTION

It is estimated that only 1% of introduced plant species will become invasive (Smith *et al.* 1999). In some regions many introduced species never become invasive due to the presence of multiple barriers (Richardson *et al.* 1992; Mack 1996), including losses caused by generalist or pre-adapted natural enemies, absence of mutualists (pollinators) and presence of novel competitors (Crawley 1987; Cronk & Fuller 1995; Richardson *et al.* 2000; Colautti *et al.* 2004). The nature of biotic interactions gained by an introduced species will depend largely on its relatedness to native species in the country of introduction (Mitchell *et al.* 2006). The arthropods and pathogens that colonize introduced plants will be related to aspects of the geographic range of the introduced species and the taxonomic, phenological, biochemical and morphological match between the introduced plant and indigenous plant species in the introduced range (Strong *et al.* 1984). Introduced species with native congeners will not necessarily experience release from natural enemies, but rather an exchange, if enemies in the invaded range elicit the same response in the host as the enemies that have been lost (Hokkanen & Pimentel 1989; Colautti *et al.* 2004).

Bryophyllum delagoense (Eckl. & Zeyh.) Schinz (Crassulaceae; mother-of-millions, finger plant) endemic to Madagascar, is a popular garden ornamental which has escaped from cultivation and become naturalized in many subtropical and tropical countries of the world, including Australia, South Africa and the United States, as have other *Bryophyllum* species (Hannan-Jones & Playford 2002). One of the reasons for its popularity can be attributed to the fact that it reproduces sexually and asexually; each leaf produces a number of plantlets or bulbils which drop off onto the ground and grow which means that species within the genus can be propagated with ease. This plant provides an excellent opportunity to test hypotheses on the role of biotic resistance in preventing or inhibiting the establishment and spread of an introduced plant species because of the phylogenetic relatedness of *B. delagoense* to other crassulaceous species in each region, and the extent of its invasion in each country differs markedly.

The date of first introduction of *B. delagoense* to Australia is unknown, but the first reported outbreak was in Chinchilla Shire, Queensland, in 1940 (Hannan-Jones & Playford 2002). It has subsequently spread rapidly and become prolific in parts of Queensland and the whole coastal strip of New South Wales. Other *Bryophyllum* species, such as *B. pinnatum* (Lam.) Oken and *B. proliferum* (Bowie) Hamet, endemic to Madagascar, are also becoming invasive in parts of Australia (Hannan-Jones & Playford 2002). In the United States, *B. delagoense* is common particularly in Florida, Texas and Hawaii (USDA 2007), but only its congener, *B. pinnatum*, also endemic to Madagascar, has been identified as a Category II invasive species in Florida (FLEPPC 2005).

The type specimen of *B. delagoense* was collected on the African mainland in Maputo, Mozambique, in the 1830s, and not in Madagascar. Subsequent to its introduction onto the African continent more than 170 years ago, *B. delagoense* has been widely disseminated as a garden plant, as were other *Bryophyllum* species, including *B. pinnatum*. Despite the long history of *B. delagoense* in southern Africa, it is not perceived as being invasive at this stage, with only eleven field infestations discovered in Botswana and South Africa, covering a total area of less than five hectares (Fig. 1). Other *Bryophyllum* species naturalized in southern Africa, such as *B. pinnatum* and *B. proliferum*, have also not reached pest status in Africa, in contrast to the situation in Australia and Florida.

This study investigates the hypothesis that biotic resistance may be preventing *B. delagoense* and some of its congeners from reaching pest status in southern Africa, compared to Australia and the United States, despite their presence on the African continent for so much longer. Competition as a biotic resistance factor is not considered in this paper, nor are abiotic factors investigated, they are considered elsewhere. This paper investigates the possible acquisition of pre-adapted natural enemies and the potential absence of specialist pollinators as biotic factors preventing *B. delagoense* from becoming invasive in Africa.

MATERIALS AND METHODS

Seed viability

To determine if successful pollination of *B. delagoense* takes place in South Africa, a comparative germination trial was undertaken using *B. delagoense* seeds collected in South Africa, Fort Dauphin in Madagascar, and Queensland, Australia. Mature seeds were collected from a number of South African sites during October and November 2004 and 2005, by gently tapping the inflorescences of a number of plants over a sheet of paper until most of the seeds had dropped out. Colleagues collected seeds using a similar method on two occasions in late 2004 at Fort Dauphin airport, Madagascar, and on one occasion at Leichardt, Australia, in 2002. Seeds from each African site, collected 2-3 months prior to the trial, were placed on moist filter paper in Petri dishes (10 cm diameter) in a growth chamber with day and night temperatures of 30°C and 20°C, respectively, and a photo-period of 12L:12D. Twenty seeds were placed in each of two Petri dishes for each site. The filter paper was moistened daily and all germinating seeds recorded and removed daily. Identical germination trials were undertaken with seeds collected in Australia and Madagascar.

Natural enemies

Information on the distribution of *B. delagoense* and other closely related introduced species in southern Africa was initially obtained from observations by staff of the Weeds Research Division of the ARC-Plant Protection Research Institute (ARC-PPRI), Pretoria, and Mr. L. Foxcroft of the Alien Biota Unit of South African National Parks, and by roadside surveys. Based on this information, systematic surveys of *Bryophyllum* species (Table 1) were undertaken in South Africa and Botswana (Fig. 1) to determine how many insect species occurred on species within this genus. *Bryophyllum* species in cultivation (gardens) were also surveyed and all associated insects collected. Sites in South Africa were surveyed at least once every four months from 1999 to 2002. Thirty-one sites with *B. delagoense* in cultivation or growing ferally were surveyed for insects in southern Africa (Fig. 1).

Table 1. Species in the genus *Bryophyllum* surveyed in South Africa and Botswana for the presence of insect natural enemies

Species	Localities surveyed	Number of surveys
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz	31	51
B. proliferum Bowie ex Hook.	3	4
B. pinnatum (Lam.) Oken	6	8
B. daigremontianum Hamet & Perrier	6	7
B. fedtschenkoi Hamet & Perrier	3	7
B. gastonis-bonnieri Hamet & Perrier	1	1

The majority of plants (c. five minutes per plant) at each locality were intensively scrutinized, and actively moving insects were captured using an aspirator or net. Sessile insects were collected with the plant part they were feeding on, while plants which appeared to have internal insect damage were removed whole and placed in emergence boxes. All insect specimens collected were submitted for identification to the South African National Collection of Insects (SANC), Pretoria, South Africa. An intensive literature survey was undertaken to obtain records of insects associated with introduced crassulaceous species, and unpublished host plant records in the SANC were examined.

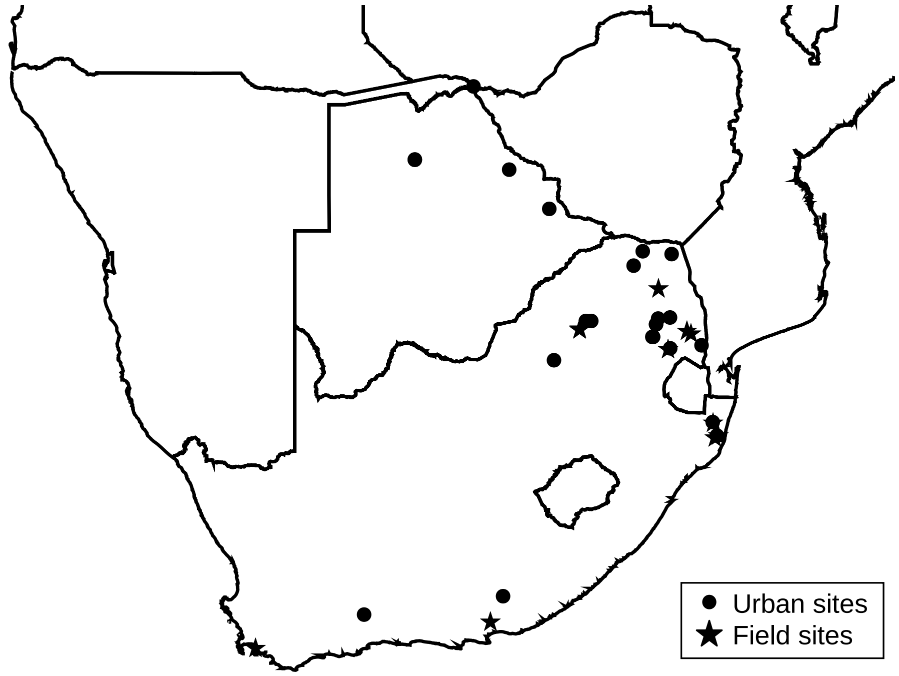

Figure 1 Bryophyllum delagoense sites in southern Africa which were surveyed for the presence of natural enemies

It was outside the scope of this study to determine the impact of each insect species collected from *B. delagoense* on plant growth or reproduction. However, data on the impact of *Alcidodes sedi* (Marshall) (Coleoptera: Curculionidae) were available from other studies (Witt *et al.* 2004b), while the impact of *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and other insect species were inferred from field observations and the literature.

RESULTS

Seed viability

A large percentage of seeds (*n* = 40 per locality) collected at one site in Madagascar (mean = 57.5%) and Australia (mean = 92.5%) and 12 localities in South Africa (mean = 50%; range = 25-100%), germinated within 50 days after the trial commenced. Although there was large variability in germination success between seeds from different sites, the results nevertheless indicate that seeds from South African *B. delagoense* are viable. A number of pollinators, including honeybees, *Apis mellifera* Linnaeus, and sunbirds (Nectariniidae) were observed visiting flowers of *B. delagoense* in Pretoria, South Africa.

Natural enemies

Most of the *B. delagoense* field infestations in southern Africa were associated with abandoned homesteads or garden refuse disposal sites. The largest field infestations in South Africa were found at widely separated sites: Sabie Park (28°01’20”S; 32°16’24”E) in Mpumalanga; near Addo Elephant National Park (33°24’34”S; 25°42’57”E) in the Eastern Cape Province; and at False Bay (27°58’01”S; 32°22’48”E) near Hluhluwe, KwaZulu-Natal province. The introduced congeners, *B. proliferum, B. daigremontianum* Hamet & Perrier and *B. pinnatum*, all endemic to Madagascar, were not as common as *B. delagoense* and, were only found at two to seven localities each, mainly in Mpumalanga. Of the insect species collected on *B. delagoense* in southern Africa during these surveys and in others (Williams 1994), fourteen were assumed to be able to complete their development on this plant species, because immature life stages were found on the plant, or they could be reared on the plant in laboratory situations (Fig. 2). Of these, *Alcidodes sedi*, *Scirtothrips aurantii* and *Aphis sedi* Kaltenbach (Hemiptera: Aphididae) were the most abundant and widespread. *Alcidodes sedi* was also collected on the introduced *B. proliferum*, and the native species *Cotyledon orbiculata* L. and *Kalanchoe sexangularis* N.E.Br. in gardens in Pretoria, Ohrigstad and Lydenburg, on the native *K. thyrsiflora* Harv. in Pretoria North (SANC unpublished record), and also in the field on the native *K. longiflora* Schltr. ex J.M. Wood in Mkhuze Game Reserve, KwaZulu-Natal.

The most conspicuous damage in the greenhouse, some gardens and occasionally in the field was caused by *S. aurantii*. Adults and nymphs feed on the epidermal cells of the leaves, particularly on new growth, causing the outer layer of the leaves to become hard and corky and resulting in severe leaf drop, reduction in bulbil production and termination of growth at infested terminal growth points. *Aphis sedi*, which was found at most sites and on many crassulaceous species surveyed, was especially abundant on the new growth and inflorescences of potted and garden plants. Another indigenous aphid species, *Aphis gossypii* Glover, was not collected on introduced *Bryophyllum* species in this study, but Millar (1994) recorded it from *Bryophyllum* species, *C. orbiculata*, *Kalanchoe brachyloba* Welw. ex Britten, *K. crenata* (Andrews) Haw. and *K. integrifolia* Baker.

Surveys and a literature review revealed that there are a number of insect species feeding on *B. delagoense* and other species in the Crassulaceae in southern Africa (Table 2). *Pandemis* cf. *catadryas* (Meyrick) was particularly damaging in some cultivated situations and on potted plants in the glasshouse but was never found in the field. *Agrotis longidentifer* (Hampson) (Lepidoptera: Noctuidae), collected on *B. delagoense* in Madagascar, also occurs on the African mainland – although it was not collected on *B. delagoense* during this survey, it should be considered a natural enemy in southern Africa. An unidentified conoderine weevil species (Curculionidae: Conoderinae) was found on *B. pinnatum* at Kampersrus (24°30’36”S; 30°53’27”E), Limpopo Province. It was tentatively placed in Coryssomerini (R. Stals, SANC, Pretoria, pers. comm.), the same tribe as *Osphilia tenuipes* (Fairmaire), a very destructive stem-boring weevil collected from *B. delagoense* in Madagascar (Witt 2004; Witt & Rajaonarison 2004).

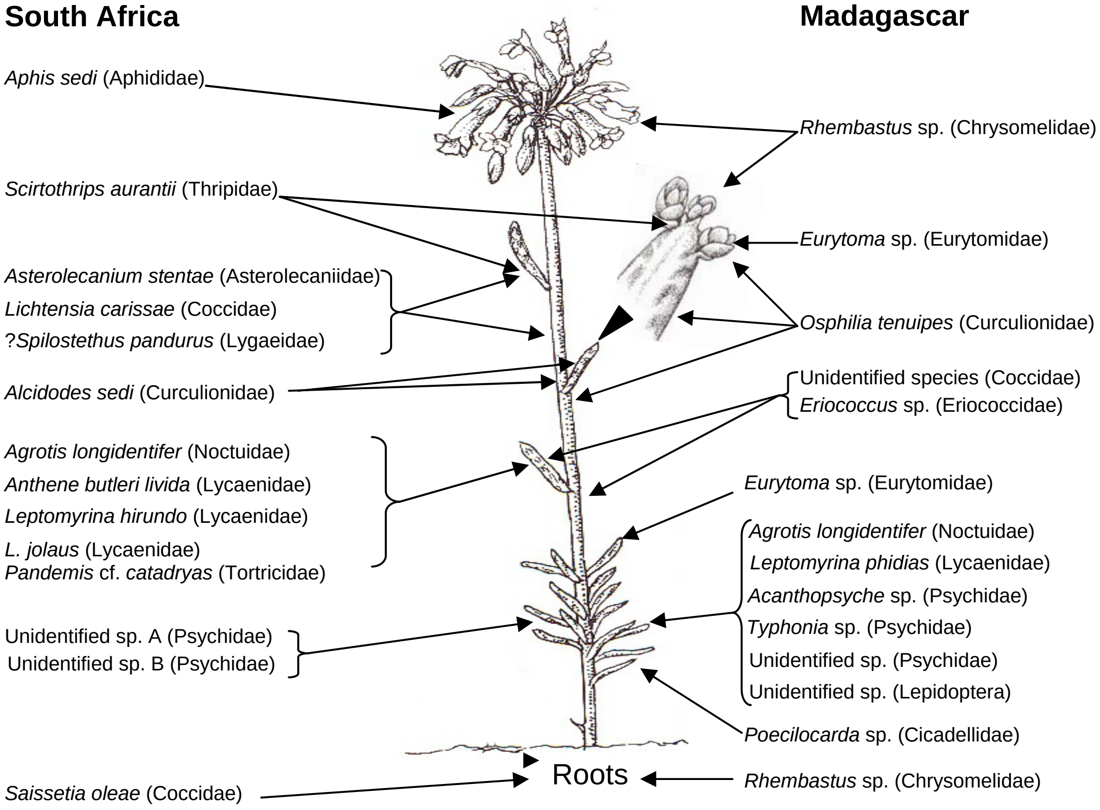

Figure 2 Phytophagous insects collected on *B. delagoense* in South Africa and Madagascar that could be positively associated with the plant. The arrows indicate which plant parts various life stages of the species of insect feed on. The records of *L. hirundo* and *L. jolaus* were obtained from Williams (1994). Line drawing by Gill Condy: in Henderson (2001).

The scale insect *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) was collected on the roots of *B. delagoense* at some sites. The most severe feeding damage on *B. delagoense* plants, similar to that caused by *A. sedi* (see Witt *et al*., 2004b), was observed at the entrance to the Steenbras Dam (34°10’32”S; 18°50’53”E) near Gordon’s Bay in the Western Cape Province, but the causative agent was never found.

Table 2. Insect species found feeding on species in the family Crassulaceae in southern Africa

Species	Host plant	Reference
LEPIDOPTERA		
Lycaenidae		
Anthene butleri livida (Trimen)	*B. delagoense*	Pers. obs.
	Cotyledon sp.	Kroon 1978; SANC unpubl. Record.
	C. orbiculata L.	Pringle et al. 1994
	Crassula sp.	SANC unpubl. Record
	Kalanchoe crenata (Andrews) Haw.	Clark & Dickson 1971
	K. lugardii Bullock	Clark & Dickson 1971
	K. blossfeldiana (von Poellnitz) Boiteau	Kroon 1999
Leptomyrina (*Leptomyrina*) *hirundo* (Wallengren)	*B. delagoense*	Williams 1994
	Cotyledon sp.	Clark & Dickson 1971
	Crassula sp.	Clark & Dickson 1971
	Kalanchoe sp.	Clark & Dickson 1971
L. (*Gonatomyrina*) *jolaus* (Cramer)	*B. delagoense*	Williams 1994
	C. orbiculata L.	Kroon 1978
	C. alba Forssk.	Pringle et al. 1994
	K. blossfeldiana (von Poellnitz) Boiteau	Kroon 1999
Tortricidae		
Pandemus cf. *catadryas* (Meyrick)	*B. delagoense*	Pers. obs.
Cryptophlebia leucotreta (Meyrick)	Crassulaceae spp.	Annecke & Moran 1982
	Crassula sp.	Taylor 1957
	C. orbiculata L.	SANC unpubl. Record
Unidentified sp.	*B. daigremontianum* Hamet & Perrier	Pers. obs.
COLEOPTERA		
Curculionidae		
Alcidodes sedi (Marshall)	*B. proliferum* (Bowie) Hamet	Pers. obs.
	B. pinnatum (Lam.) Oken	Pers. obs.
	B. delagoense	
Unidentified Conoderinae sp.	*B. pinnatum* (Lam.) Oken	Pers. Obs
THYSANOPTERA		
Thripidae		
Unidentified sp.	*B. pinnatum* (Lam.) Oken	Pers. obs.
	B. daigremontianum Hamet & Perrier	Pers. obs.

DISCUSSION

The study of insect-host plant associations, like all historical ecology, is incomplete without a phylogeny (e.g. Brooks & McLennan 2002). Two recent molecular phylogenetic studies investigated the intrafamilial relationships of the Crassulaceae and recovered the same basal dichotomy, leading to two monophyletic subfamilies, namely Crassuloideae, containing only the genus *Crassula sensu lato*, and Sedoideae, comprising the remainder of the genera (Van Ham and 't'Hart 1998; Mort *et al.* 2001; see Fig. 3). The genus *Crassula* hence is sister to the rest of the Crassulaceae. The species of *Crassula* occur predominantly in southern Africa (Jürgens 1995). Within the Sedoideae, six clades are apparent, one of which is a strongly supported *Kalanchoe* clade, consisting of the six genera *Adromischus*, *Bryophyllum*, *Cotyledon*, *Kalanchoe*, *Kitchingia* and *Tylecodon* (Mort *et al.* 2001). The *Kalanchoe* clade, mostly distributed in southern Africa (Eggli *et al.* 1995), is sister to the remainder of the Sedoideae, which includes, among others, the large genera *Sedum* and *Echeveria* that dominate the American crassulaceous flora (Thiede 1995). Although the Americas have more than 400 Crassulaceae species, no genus of the *Kalanchoe* clade is native there (Thiede 1995). Australia has only eight indigenous species of Crassulaceae, all belonging to the genus *Crassula* (Tölken 1981) with one possible exception. *Kalanchoe crenata* has been recorded from north-western Western Australia, but it is unclear whether this species is native or introduced to Australia (Kenneally 1983). It is not endemic to Australia, as it also occurs throughout Africa and Asia (Kenneally 1983) and is probably adventive in Australia. Here, *K. crenata* is considered to be introduced to Australia. Sedoideae, including the *Kalanchoe* clade, are thus absent from the native Australian flora.

In North America, introduced plants that experienced more herbivory (Carpenter & Cappuccino 2005) or acquired more pathogens (Mitchell & Power, 2003) were less frequently reported as invasive. The insects and pathogens associated with native Crassulaceae may be able to expand their host ranges to include closely related introduced species and thereby prevent or inhibit the alien species from becoming invasive (Mack 1996). In southern Africa there are a large number of plant species taxonomically related to *Bryophyllum* species inasmuch they also belong to the *Kalanchoe* clade. Burgoyne and Bruyns (2003) listed 98 species in the genera *Adromischus*, *Cotyledon*, *Kalanchoe* and *Tylecodon* native to southern Africa. The pool of insects and pathogens potentially pre-adapted to feed and develop on introduced *Bryophyllum* species is therefore hypothetically larger in southern Africa, where the *Kalanchoe* clade is native and diverse, than in Australia, where the *Kalanchoe* clade is absent. Similarly,

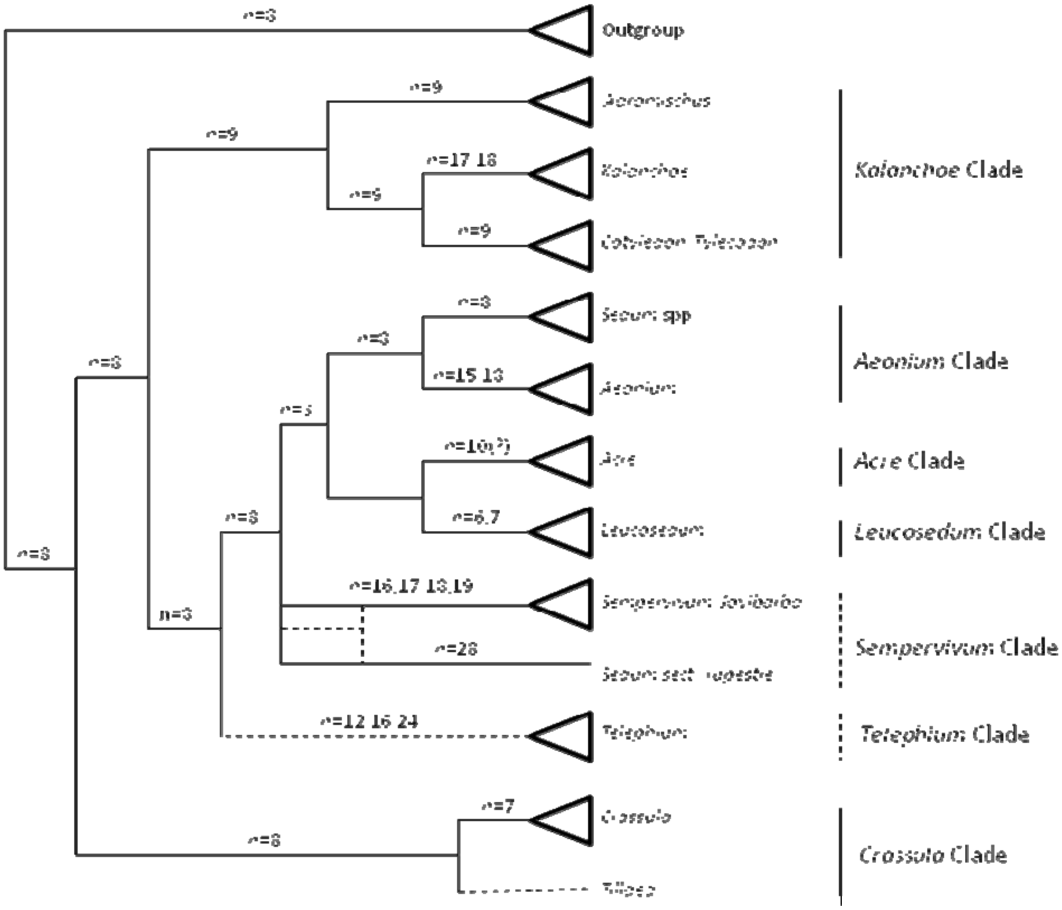

Figure 3 Simplified summary topology representing the relationships among major clades in the Crassulaceae. Base chromosome numbers reconstructed using MacClade are indicated above each branch. Adapted from Mort *et al*. (2001).

because of the placement of *Bryophyllum* in the Sedoideae, this may also hold for Mexico and the United States, *versus* Australia, where members of the Sedoideae are absent. Only the Crassuloideae is represented in the native Australian flora, more distantly related to *Bryophyllum*. There are also no indigenous crassulaceous species in Florida (KD Perkins, University of Florida Herbarium, Florida Museum of Natural History, personal communication, 2006), where *B. pinnatum* has been declared a Category II invasive species (FLEPPC 2005).

According to the literature survey, combined with the results of the field surveys, most of the insects collected on *B. delagoense* in southern Africa have a host range which predominantly includes species in the genera *Cotyledon* and *Kalanchoe*. There are fewer records of these very same insect species also occurring on species in the genera *Crassula* and *Echeveria*, while a much smaller number also feed on plants outside of the Crassulaceae. This is in contrast to three potential biocontrol agents collected on *B. delagoense* in Madagascar, whose preferred hosts in laboratory host range trials were only species in the genera *Bryophyllum* and *Kalanchoe* (Witt 2004; Witt *et al.* 2004a, 2006). *Osphilia tenuipes* could develop on an *Echeveria* species in host range trials in South Africa, albeit in very low numbers (Witt 2004), while statistically fewer adults emerged from *Sedum praealtum* D.C. and *S. morganianum* Walther in no-choice trials in Australia (WA Palmer, Queensland Department of Primary Industries, personal communication). The results indicate that these species are not suitable hosts for *O. tenuipes* and are unlikely to be attacked in the field. *Alcidodes sedi*, which has also undergone preliminary laboratory host range trials, could only complete its development on species in the genera *Kalanchoe*, *Cotyledon* and *Bryophyllum*, all in the *Kalanchoe* clade, and not on *Crassula* species (Witt *et al.* 2004b). A very small number of adults emerged from an *Echeveria* hybrid during laboratory trials but this was considered unlikely to happen in the field (Witt et al 2004b). The host ranges of these three Madagascan insect species and of *Alcidodes sedi* are therefore to a large extent congruent with the intrafamilial phylogenetic relationships of the Crassulaceae as hypothesised by Van Ham and ‘t Hart (1998) and Mort *et al.* (2001).

Despite a number of records of native Lepidoptera developing on indigenous crassulaceous species in the USA, there are no records of any species developing on introduced *Bryophyllum* species (Pyle 2002; Scott 1986). Phylogenetic studies have demonstrated that related butterflies are more likely to feed on related plants (Janz & Nylin 1998), but *Bryophyllum* species introduced to America may be too distantly related to the native *Echeveria* and *Sedum* species to be suitable hosts for native butterflies and other insect species. The ability of some insect species collected on *B. delegoense* to develop on *Echeveria* and *Sedum* species in laboratory trials, albeit at lower levels compared to the target species (Witt 2004; Witt *et al.* 2004a, 2004b, 2006), is an indication that the American crassulaceous species are more closely related to species in the *Kalanchoe* clade than the crassulaceous flora in Australia. Surveys in Australia have only found two species of insects, both of them introduced, *Scirtothrips aurantii* and *Aphis sedi*, which can complete their development on *B. delagoense* (MA Hannan-Jones, Queensland Department of Primary Industries & Forestry, personal communication). *Scirtothrips aurantii*, a polyphagous pest native to southern Africa, inflicting heavy feeding damage to a range of crops and indigenous plant species (Gilbert & Bedford 1998), was first recorded in Australia in 2002 after its unintentional introduction, probably some years earlier (Manners & Dhileepan 2005; Palmer 2005). However, studies in Australia have indicated that the *S. aurantii* in Australia does not develop on citrus, mangos and other species, but appears to be restricted to species in the Crassulaceae (Manners and Dhileepan 2005). This may be an indication that *S. aurantii* consists of different biotypes with restricted host ranges, although a preliminary molecular analysis failed to show that population structure in *S. aurantii* is directly linked to host plant affiliation (Morris & Mound 2004). The damage caused by *S. aurantii* to *B. delagoense*, may very well inhibit the further spread of *B. delagoense* in Australia.

Indigenous natural enemies pre-adapted to feed on an introduced plant species related to their native host plant will be more likely to attack the introduced plant species should it establish within the natural extent of occurrence of related native species (Strong *et al.* 1984). Vast distances and natural barriers such as mountain ranges or deserts may prevent indigenous natural enemies from reaching and establishing on introduced species. There are no indigenous crassulaceous species in Florida, USA, or in central and northern Queensland, Australia (Tölken 1981) (Fig. 4). Very few *Crassula* species extend their natural ranges into Queensland (Tölken 1981), a region where *B. delagoense* is particularly invasive (Hannan-Jones & Playford 2002). In addition, most of the *Crassula* species in Australia are annuals (Tölken 1981) which may therefore have a more depauperate insect fauna (Lawton & Schroder 1977). The absence of closely related Crassulaceae and their associated natural enemies, and the allopatric distribution of the introduced *Bryophyllum* species and native Crassulaceae, may be contributing to the strong invasiveness of *Bryophyllum* species in Australia. Long term monitoring of transplanted plants would be useful to test this hypothesis (McFadyen 1998).

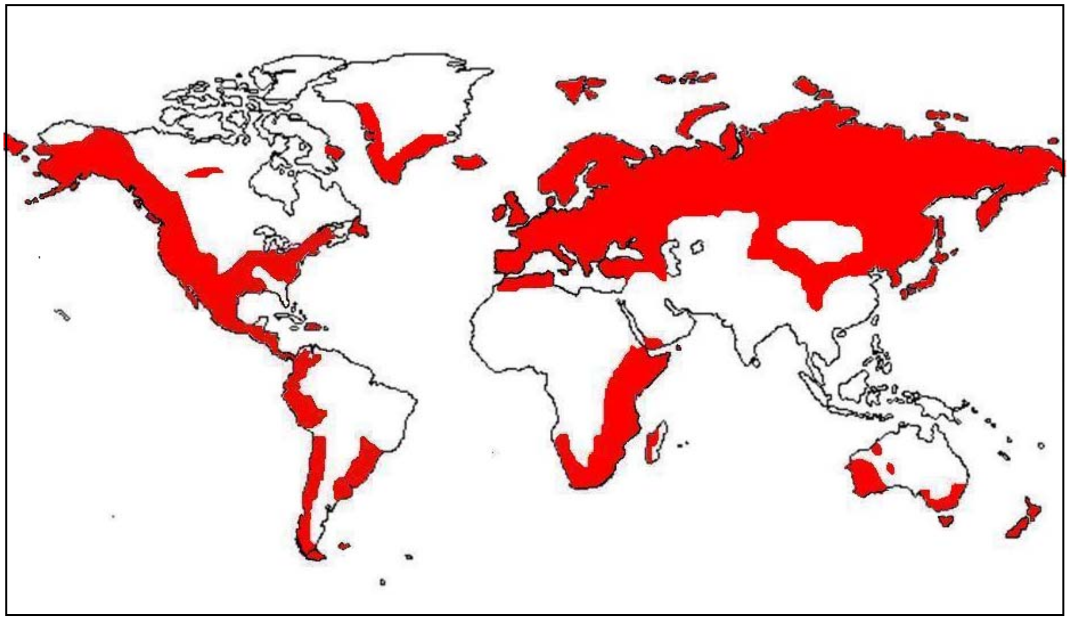

Figure 4 Worldwide distribution (red shading) of species in the family Crassulaceae (distributions of introduced species excluded) (MOBOT; no date)

Despite the fact that *B. delagoense* has the ability to reproduce asexually through the production of plantlets at the ends of its leaves, these are probably only involved in short distance dispersal. This is an effective strategy for the exclusion of interspecific competitors through site occupancy (Herrera *et al*., 2011). According to Judd *et al.* (1999) and Thiede (1995), the tiny seeds of Crassulaceae species are probably wind dispersed. The thousands of tiny seeds produced by *B. delagoense* may therefore be involved in long distance disperal. Despite the large size of the family Crassulaceae, very little is known about its reproductive biology and pollination ecology (Zietsman 1998). According to early studies by Muller (1883) and Knuth (1908), most of the species are probably pollinated by generalist insects. Para *et al.* (1993) found that *Echeveria gibbiflora* D.C. was only pollinated by one species of hummingbird (Trochilidae), while Levin and Mulroy (1985) determined that one group of *Dudleya* species was pollinated by bees and long-tongued flies, another by hummingbirds, and a third group was autogamous. In South Africa, pollination of *Cotyledon orbiculata* var. *dactylopsis* Tölken was achieved in the absence of sunbirds, which were considered opportunistic visitors and not specialized or reliable pollinators (Zietsman 1998). *Bryophyllum daigremontiana* is autogamous and was characterized by low flower visitation in its introduced range in Venezuela (Herrera & Nassar 2009). Floral visitors included two species of wasps and a honeybee species; no hummingbirds visited the flowers (Herrera & Nassar 2009) in contrast to what was observed in South Africa and Madagascar where sunbirds were seen on *B. delagoense* flowers. As in South Africa honeybees were also observed visiting flowers in Australia (MA Hannan-Jones, Queensland Department of Primary Industries & Forestry, personal communication). Nevertheless, despite the low visitation levels the germination trial indicated a high level of pollination success in Australia and South Africa, and it is apparent that *B. delagoense* does not require the services of a specialist pollinator. This is in agreement with Richardson *et al.* (2000) that many pollinators are generalists. However, the possibility of *B. delagoense* being autogamous cannot be dismissed. The absence of viable *B. delagoense* seeds in southern Africa can therefore no longer be considered as a barrier to its invasion potential in the region. However, the importance of seed production in the invasion success of *B. delagoense* should be considered against the findings of Herrera *et al.* (2011) who found that the contribution of autogamy to the establishment success of *B. daigremontiana* was negligible. Plantlets of asexual origin showed high survival (75-100%) compared to seedlings of sexual origin (10%) (Herrera & Nassar 2009).

Abiotic factors such as climate, soil types and infrequency of major disturbance events (e.g. fire) (Richardson *et al.* 1992; Mack 1996) have been considered elsewhere as barriers to invasion by *B. delagoense* in southern Africa (Witt, Nongogo & Byrne submitted) and show the plant to be tolerant of a wide range of conditions. Therefore, we conclude that the recruitment of pre-adapted natural enemies may be inhibiting *B. delagoense* from reaching pest status in this region. In addition, it has been hypothesized (Evolution of Increased Competitive Ability) that exotics long liberated from their natural enemies should lose costly traits that confer resistance to their native natural enemies and reallocate those resources to growth and fecundity (Blossey & Nötzold 1995). It is therefore predicted that liberation from natural enemies should result in exotics evolving in ways that enhance their performance. This may also be contributing to the increased invasiveness of *B. delagoense* in Australia compared to South Africa. However, we also recognise that some species may have a very long lag-period before they become invasive (Kowarik 1995), and therefore support the South African authorities in applying the precautionary principle, based on the biological traits of *B. delagoense* and its invasion history elsewhere in the world, in that this plant is listed as a prohibited species under the current regulations of the Conservation of Agricultural Resources Act (Henderson 2001).

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CHAPTER 8

ALCIDODES SEDI (COL.: CURCULIONIDAE), A NATURAL ENEMY OF *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE) IN SOUTH AFRICA AND A POSSIBLE CANDIDATE AGENT FOR THE BIOLOGICAL CONTROL OF THIS WEED IN AUSTRALIA

Justification

This chapter follows-on from the preceding ones where we examined the natural enemies associated with *B. delagoense* in its native range and then undertook trials to determine the biology and host range of three selected species of these insect herbivores with the potential to be biological control agents of this weed in Australia. This was followed by surveys of natural enemies on *B. delagoense* in southern Africa where the plant has been present for over 170 years. The search was based on the theory of "new associations" as promulgated by Hokkanen and Pimentel (1984). Support for this theory has also come from Dennill and Moran (1989) who found that 58% of the 40 most important agricultural pests in South Africa are in "new associations". Many invasive plants present in South Africa have recruited indigenous insects and pathogens (Holmes *et al*., 1987; Morris, 1983). This supports the hypothesis that invading species form new relationships with species in the invaded community, which may impede the success of a potential invader (Mitchell *et al*., 2006). It is more likely that an introduced species that is more closely related to the native species will acquire more natural enemies (Lawton and Schroder, 1977; Strauss *et al*., 2006). Surveys in southern Africa revealed a number of natural enemies associated with *B. delagoense* (Witt, McConnachie and Stals submitted). One of these natural enemies, *Alcidodes sedi*, was selected for further studies because it was widespread, relatively abundant in the field, very damaging, and based on field surveys appeared to be restricted to species in the genus *Bryophyllum* and *Kalanchoe*. This would not preclude it from release in Australia which has only eight indigenous species of Crassulaceae, all in the genus *Crassula* (Tölken, 1981) with one possible exception. It is unknown if *K. crenata* is native or introduced to Australia (see Kenneally, 1983) but agents released on *B. delagoense* would be geographically isolated from *K. crenata* populations in north-western Western Australia anyway.

Alcidodes sedi is the fourth agent proposed for the biocontrol of *B. delagoense* in Australia – supporting the contention that the highest levels of biocontrol 'success' will be achieved using multiple agents (Denoth *et al*., 2002; Harris, 1981; Hoffmann and Moran, 1998). However, there may be direct competition (Ehler and Hall, 1982) between *A. sedi* and *Osphilia tenuipes* because the larvae of both species develop in the stems *B. delagoense*. This may not necessarily preclude one of the two species if they are active under different climatic regimes or if they can occur sympatrically – *O. tenuipes* is a smaller weevil and can, unlike *A. sedi*, develop in smaller stems. Laboratory trials have also indicated that *O. tenuipes* is also a better disperser and more likely to find isolated infestations. It is important to resolve all of these issues prior to release, to avoid releasing unnecessary agents, each of which will represent a possible threat to recipient ecosystems, however small that threat might be (McEvoy and Coombs, 2000).

This chapter is novel in that it decribes the biology, host range and impact of a native insect which is pre-adapted to feed on an introduced plant, *B. delagoense*, in South Africa, and as such is being considered for the biocontrol of this weed in Australia. It was not selected for testing based on the premise, as proposed by Hokkanen and Pimentel (1984), that being a "new association" it would be a more effective biocontrol agent than, for example, *O. tenuipes*. *Alcidodes sedi* was relatively easy to rear under laboratory conditions and completed its development in about 30 days. Adult feeding and larval development retarded stem growth and caused a significant reduction in the number of leaves and weights of test plants. In no-choice trials *A. sedi* developed on six of the nine *Bryophyllum* and *Kalanchoe* species but not on any of the *Crassula* species tested, which increases its potential as an agent for release in Australia, if the question of competition between it and *O. tenuipes* can be resolved. Because *A. sedi* presents a biotic pressure on the plant in southern Africa, which could be suppressing the invasive potential of the weed, this chapter is followed by further investigation of other biotic and abiotic factors which affect *B. delagoense*, to further evaluate why this weed is so invasive in Australia and not in southern Africa.

Andrew McConnachie was the technician who assisted with surveys while Riaan Stals from the National Collection of Insects (ARC-PPRI) provided information on the taxonomy of *A. sedi*. This paper was published in the journal Biological Control 31: 380-387 (2004).

***Alcidodes sedi* (Col.: Curculionidae), a natural enemy of *Bryophyllum delagoense* (Crassulaceae) in South Africa and a possible candidate agent for the biological control of this weed in Australia**

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Abstract

The Madagascan endemic, *Bryophyllum delagoense* (Crassulaceae), is a major weed in Queensland, Australia. Despite having first been recorded in Australia in the 1940s, it is far more invasive there than on the African mainland where it was introduced more than 170 years ago. This may be due to a number of factors, one of which could be the occurrence of new natural enemy associations in southern Africa. Among the insects of crassulaceous plants that have extended their host ranges, a stem-boring weevil, *Alcidodes sedi,* was studied to elucidate its status as a natural enemy of *B. delagoense* in southern Africa and as a candidate biological control agent for introduction to Australia. Laboratory studies indicated that damage inflicted by adult and larval feeding caused significant reductions in stem length and number of leaves. Preliminary host-range trials revealed that *A. sedi* can complete its development on other species in the Crassulaceae, including most of the introduced *Bryophyllum* species and some *Kalanchoe* species native to South Africa. Despite the oligophagous nature of *A. sedi* and the fact that it can complete its development on a number of ornamental species in the Crassulaceae, it should be considered a potential biological control agent in Australia. All of the native Crassulaceae in Australia are in the genus *Crassula*, most of which are very small and therefore unlikely to support the development of a large weevil like *A. sedi*. However, additional host-range trials will have to be undertaken in Australia to determine whether the weevil can be considered safe for release.

Keywords: *Bryophyllum delagoense*; Crassulaceae; Biological control; *Alcidodes sedi*; Curculionidae; Host range.

1. Introduction

Bryophyllum delagoense (Eckl. & Zeyh.) Schinz (Crassulaceae, mother-of-millions), an introduced plant from Madagascar, was first recorded in southern Africa in the 1830s and in Australia in the 1940s (Hannan-Jones and Playford, 2002). Since its introduction into Australia, it has invaded thousands of hectares of grazing land (Hannan-Jones and Playford, 2002; A.B.R. Witt, personal observation), whereas it is not currently regarded a serious problem in southern Africa. Based on its current status in Australia, there is concern in southern Africa that it may become problematic in future (Henderson, 2001). Characteristics which contribute to its invasiveness are the plantlets (bulbils), produced on its succulent leaves, which establish by dropping to the ground, and the fact that each inflorescence produces thousands of seeds, most of which are viable (Hannan-Jones and Playford, 2002). The plant is also poisonous, containing bufadienolide glycosides, and has caused livestock losses in Australia (Capon et al., 1986). Established infestations are difficult and expensive to eradicate mechanically or chemically. Because this plant has few natural enemies in Australia (M. Hannan-Jones, Queensland Department of Natural Resources & Mines, Australia, personal communication), surveys for potential biological control agents were undertaken in Madagascar (Witt and Rajaonarison, 2004). Surveys were also undertaken in South Africa and Botswana to ascertain if any native arthropods or pathogens associated with native Crassulaceae had extended their host ranges to include *B. delagoense*. Such organisms could also have potential as biocontrol agents in Australia.

A number of promising insect species were collected on *B. delagoense* in Madagascar, three of which, including a stem-boring weevil, *Osphilia tenuipes* (Fairmaire) (Coleoptera: Curculionidae: Conoderinae: Coryssomerini) were selected as potential biocontrol agents (Witt and Rajaonarison, 2004). Preliminary host-range testing revealed that all three species could complete their development on some species of Crassulaceae native to South Africa. This indicated that although these insect species could well be considered as biocontrol agents for release in Australia, they cannot be released on the African mainland. During surveys in South Africa and Botswana, a native weevil, *Alcidodes sedi* (Marshall) (Coleoptera: Curculionidae: Molytinae: Mecysolobini), was found to have extended its host range to include *B. delagoense*. It is currently considered to be an additional potential agent for release in Australia and possibly for augmentative releases at isolated infestations in South Africa.

The weevil tribe Mecysolobini in which *A. sedi* has been placed is in need of comprehensive, modern revision and the generic placement of many mecysolobine species should presently be considered tentative. Haaf (1961), who last reviewed the Afro-tropical *Alcidodes sensu lato*, disregarded the confusing and contradicting classifications of previous authors (summarised by Pajni and

Dhir (1987) and Lyal and Curran (2000)) and applied the genus *Alcidodes* for all the species he treated. Alonso-Zarazaga and Lyal (1999) listed the 19 available genus group names in the Mecysolobini, and treated *Alcidodes* Marshall as a subgenus of *Sternuchopsis* Heller, apparently following Voss (1956). The species *A. sedi* Marshall has never been formally transferred to the latter genus. Following Lyal (1996) and Lyal and Curran (2000), we retain the use of the genus name *Alcidodes* in the interest of nomenclatural stability. In the absence of a robust phylogenetic revision of the group, *Alcidodes* should presently be considered a "holding genus" for *sedi*.

The current taxonomic status of *A. sedi* should not detract from its potential as a biocontrol agent as it causes considerable damage to *B. delagoense* plants. Adult weevils are fairly large and feed externally on the leaves and stem, while the larvae are stem-borers. In the biological control of invasive plants, weevils have been used extensively, and in many cases with great success, (Julien and Griffiths, 1998). This paper examines (i) the weevil's status as an established natural enemy of *B. delagoense* in southern Africa and (ii) the potential of using *A. sedi*, a "new association" as promulgated by Hokkanen and Pimentel (1984), as a classical biological control agent in Australia. In addressing these issues, aspects of the weevil's natural distribution, biology, and host range were investigated.

2. Materials and methods

2.1. Field Observations

2.1.1. Distribution.

The distribution of *A. sedi* was determined from specimens housed in the Natural History Museum (Britain), South African National Collection of Insects (ARC-PPRI), and the Transvaal Museum, both in Pretoria, South Africa. Known *B. delagoense* sites in southern Africa were surveyed during the summer of 2002/2003 for the presence of adults, larvae, and/or weevil damage. Other *Bryophyllum* species, *Kalanchoe sexangularis* N.E.Br., *K. longiflora* Schltr. ex J.M. Wood, and *Cotyledon orbiculata* L. (all Crassulaceae) were also surveyed at a number of localities at the same time (see Table 1). Other species of Crassulaceae, including the above-mentioned species, growing in association with *B. delagoense* in cultivation (i.e., in residential parks and gardens), were also examined for the presence of *A. sedi*.

2.2. Biology

2.2.1. Rearing.

Stems containing *A. sedi* larvae were collected at various sites in the Limpopo, Mpumalanga, and Gauteng provinces of South Africa (Fig. 1). Infested stems were placed in emergence boxes in the laboratory at the South African Field Station, Queensland Department of Natural Resources & Mines, based at ARC-PPRI in Pretoria. Newly emerged adults were removed and placed in large cages (650 x 400 x 430 mm) on potted *B. delagoense* plants in a glasshouse under natural light, at temperatures ranging from 14-30°C and at relative humidity ranging from 25-60%.

2.2.2. Development.

Ten unsexed adult weevils were placed for 48 h in each of 30 sleeves on potted *B. delagoense* plants. Each sleeve consisted of an empty 2-liter plastic bottle with the bottom end cut off and with gauze-covered openings for ventilation. The bottom end was stoppered with a high-density foam disc, which had a slit cut into it to allow for the placement of the plant stem. About 160 mm of each plant stem was exposed to the 10 adults. One to three sleeved plants were dissected every three to five days after the adults had been removed. The numbers of each developmental stage present were recorded. The dimensions of eggs (length and width), larvae (body length and head-capsule width), pupae (body length), and adults (body length measured from tip of abdomen to base of rostrum and maximum pronotal width) found in dissected stems were measured with an ocular micrometer fitted on an Olympus SZX9 microscope. Adults were also weighed using an electronic balance. The head-capsule widths of larvae were subjected to cluster analysis to determine the number of larval instars by using GenStat for Windows (Anonymous, 2000; Payne et al., 1989). These trials were conducted in a controlled environment room with a photoperiod of 14h L: 10h D, mean (±SD) temperature of 26 ± 1.7°C (range = 23-28°C), and mean (±SD) relative humidity of 79 ± 7% (range = 58-96%).

2.2.3. Feeding damage.

Twenty similarly sized *B. delagoense* plants were removed from a field site without weevils and planted in pots. Trials commenced 60 days later to allow for plant establishment. Stem height, stem diameter, and numbers of leaves were recorded for each of the plants immediately prior to the commencement of trials. Stem diameter for each plant was calculated based on the mean measurement of the stem at the base, middle, and top.

A single pair of copulating *A. sedi* adults was placed in each of 10 sleeves on 10 of the potted plants. The remaining 10 similarly sized plants were also sleeved, but with no weevils included. Adults were removed from plants after 10 days and identical measurements of the plants taken as those prior to exposure to adult weevils. Subsequent larval damage, without adult feeding, was assessed at the termination of the trial, 20 days after the adults had been removed. Comparisons between the control plants and plants that had been exposed to adult and subsequent larval feeding were then made based on differences in mean stem height, stem diameter, number of leaves, and wet weight. Trials were undertaken in a controlled environment room at temperatures ranging from 23-28°C and 58-96% relative humidity.

The data were analyzed using the statistical program GenStat for Windows (Anonymous, 2000). Student's *t*-test was used to test for differences between control plants and plants exposed to adult and larval feeding. The sample size of experimental plants was reduced from 10 to five because no larval development occurred in five of the experimental plants.

2.2.4. Dispersal ability.

During surveys and laboratory trials, adult weevils gave no indication that they could fly. To ascertain if they would fly under conditions of food deprivation and heat stress, 40 adult weevils were randomly collected from the rearing cage and divided into two groups of 20 each. One group was placed in a large container on *B. delagoense* plants, while the other was placed in an empty container. After seven days, 10 weevils from each container were removed and individuals placed on a hotplate in an open ring, which would allow them to fly off but prevent them from walking off. The hotplate surface was covered with a single paper towel layer to prevent possible tarsal damage and set at a temperature ranging from 40-50°C. Adults were removed if they had not flown off after 60 s. This experiment was repeated with the remaining 20 adults after 10 days.

2.2.5. Survival and longevity.

Adult longevity and survival were assessed simultaneously by comparing adults in ventilated jars containing cut *B. delagoense* (*n* = 20) stems with those kept in empty jars (*n* = 10). A single, unsexed, newly emerged adult (within 24 h of eclosion) was placed in each of 10 jars for each treatment and survival was monitored every 48 h until the last adult had died. Cut stems were replaced every five days. Jars were placed in a controlled environment room with mean (±SD) temperature of 25 ± 1.7°C (range = 23-27°C) and mean (±SD) relative humidity of 50 ± 6% (range = 30-70%).

2.3. Host Specificity

Eighteen plant species, including 16 species in the Crassulaceae and members of two other families with similar external morphology (succulent stems or leaves) were tested to determine the laboratory host range of *A. sedi*. In addition, related plants, including five additional species of *Bryophyllum* and six other species in the Crassulaceae, were examined in the field for the presence of *A. sedi* adults, larvae, and/or damage (see Table 1).

2.3.1. No-choice trials.

Sixteen species in the Crassulaceae and one each in Asteraceae and Portulacaceae were tested in no-choice trials. Since live adult weevils could not be sexed, 10 adults were collected randomly from rearing cages and were placed in each cage (650 x 400 x 430 mm) on a single plant. Three to nine different species were tested in each trial, including *B. delagoense* used as the control. Each trial was replicated three times. Trials were conducted in a glasshouse under natural light with temperatures ranging from a mean (±SD) minimum of 15 ± 2°C to a mean (±SD) maximum of 30 ± 3°C. Relative humidity ranged from a mean minimum (±SD) of 30 ± 6.7% to a mean (±SD) maximum of 66.4 ± 13.3%.

Live adult weevils were removed from each cage after 10 days. Adult feeding damage of the leaves and stem, based on the percentage of the plant damaged, was categorized as none, negligible (<25%), moderate (25-50%), or substantial (>50). All trial plants were thereafter monitored daily for adult emergence and dissected 40 days after the first adult had emerged from a plant used in that trial. The numbers of larvae, pupae, and/or adults in each stem were recorded.

3. Results

3.1. Field Observations

3.1.1. Distribution.

Specimens housed in the National Collection of Insects and the Transvaal Museum were collected in the Gauteng, North-West, Mpumalanga, and Kwazulu-Natal Provinces of South Africa. Voucher specimens of the adults collected during this survey have been lodged with the National Collection of Insects.

Alcidodes sedi larvae and/or adults were found at 20 of the 31 *B. delagoense* sites surveyed in South Africa and Botswana during this study (Table 1). The weevil was also collected in the field or in cultivation on *B. proliferum*, *B. pinnatum*, *B. daigremontianum*, *K. longiflora*, *C. orbiculata*, and *Kalanchoe sexangularis* (Table 1). Of the native species surveyed in the field *A. sedi* was only found on *K. longiflora* indicating that this species is possibly the primary host. Based on this study and data from specimens housed in other collections, the weevil has a relatively wide distribution, at least extending from Shashe Dam (21°22'01"S; 27°26'22"E) near Francistown, Botswana in the north and west to Empangeni (28°42'S; 31°46'E) in KwaZulu-Natal Province, South Africa in the south and east (Fig. 1).

Table 1

Plant species in the Crassulaceae surveyed in South Africa and Botswana for the presence of *Alcidodes sedi*.

Species	Localities surveyed	Number of surveys	*A. sedi* present[b]
Bryophyllum delagoense (Eckl. & Zeyh.) Schinz[a]	31	51	20(64.5%)
B. proliferum Bowie ex Hook.[a]	3	4	2(66.6%)
B. pinnatum (Lam.) Oken[a]	6	8	2(33.3%)
B. daigremontianum Hamet & Perrier[a]	6	7	1(16.6%)
B. fedtschenkoi Hamet & Perrier[a]	3	7	0
B. gastonis-bonnieri Hamet & Perrier[a]	1	1	0
Cotyledon orbiculata L.	17	17	3(17.6%)
Crassula ovata (Mill.) Druce	1	1	0
C. rupestris Thunb.	1	1	0
Crassula sp.	1	1	0
K. longiflora Schltr. ex J.M. Wood	2	2	2(100%)
Kalanchoe sexangularis N.E.Br	6	6	1(16.6%)

[a] Plant species native to Madagascar, but which are naturalized in southern Africa.

[b] Number of localities surveyed where *A. sedi* was present.

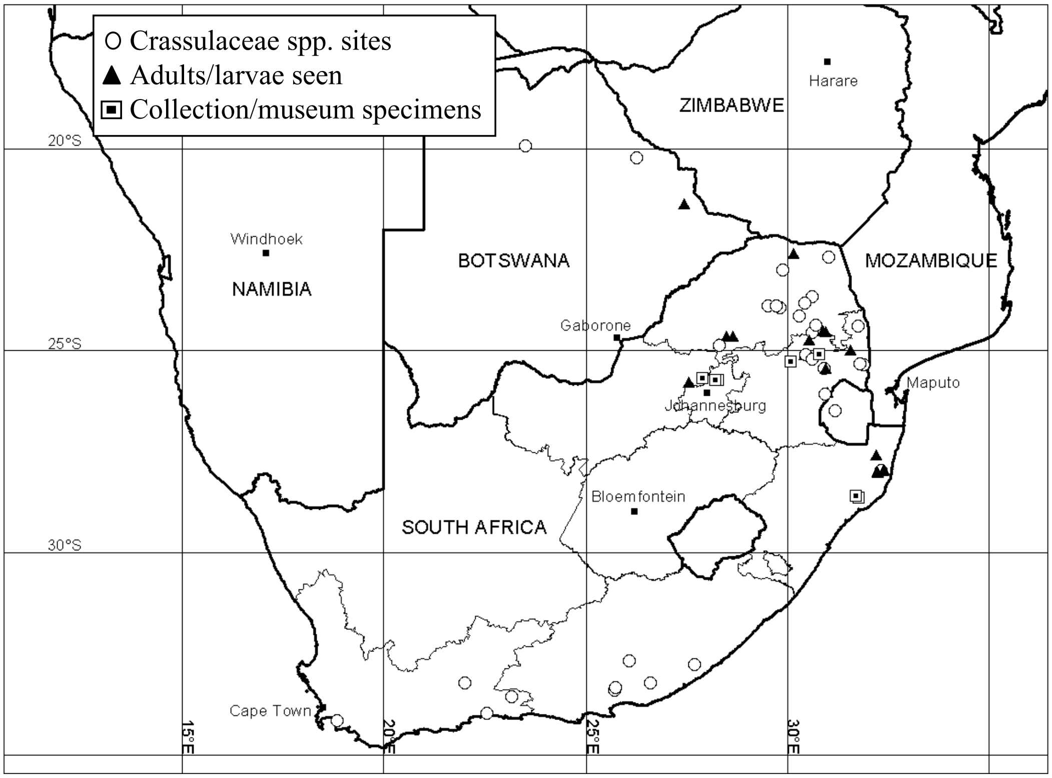

Fig. 1. Map of southern Africa showing sites where species in the Crassulaceae were surveyed, the localities where *Alcidodes sedi* adults and/or larvae were seen, and the locality data from specimens housed in the National Collection of Insects and the Transvaal Museum, Pretoria, South Africa.

3.2. Biology

3.2.1. Development.

Eggs were laid singly under the epidermis along the stem. They were white, ellipsoidal, and had a mean (±SE) length of 1.3 ± 0.04 mm and mean (±SE) width of 0.9 ± 0.02 mm (*n* = 13). Some of the stems dissected three days after having been exposed to adult weevils for 48 h contained both eggs and first instar larvae, indicating that the egg incubation period can be five days or less (Fig. 2).

Cluster analysis of head-capsule widths suggested that there are three larval instars (Fig. 3). First instar larvae had a mean (±SE) head-capsule width of 0.6 ± 0.01 mm (*n* = 15) and a mean (±SE) body length of 2.5 ± 0.06 mm (*n* = 14). First instar larvae were found directly beneath adult feeding lesions. Third instar larvae had a mean (±SE) head-capsule width of 1.6 ± 0.02 mm (*n* = 25) (Fig 3). The number of larvae in stems decreased as they matured, which could be due to a number of factors including intraspecific competition or cannibalism. Stems dissected five days after adult exposure had 10-13 first instar larvae while stems dissected after 25 days had 4-5 final instar larvae.

Pupae were dissected from stems from 24 days after adult exposure (Fig. 2) and had a mean (±SE) body length of 8.0 ± 0.3 mm (*n* = 12). Pupae were initially pale in color and turned black just prior to adult emergence.

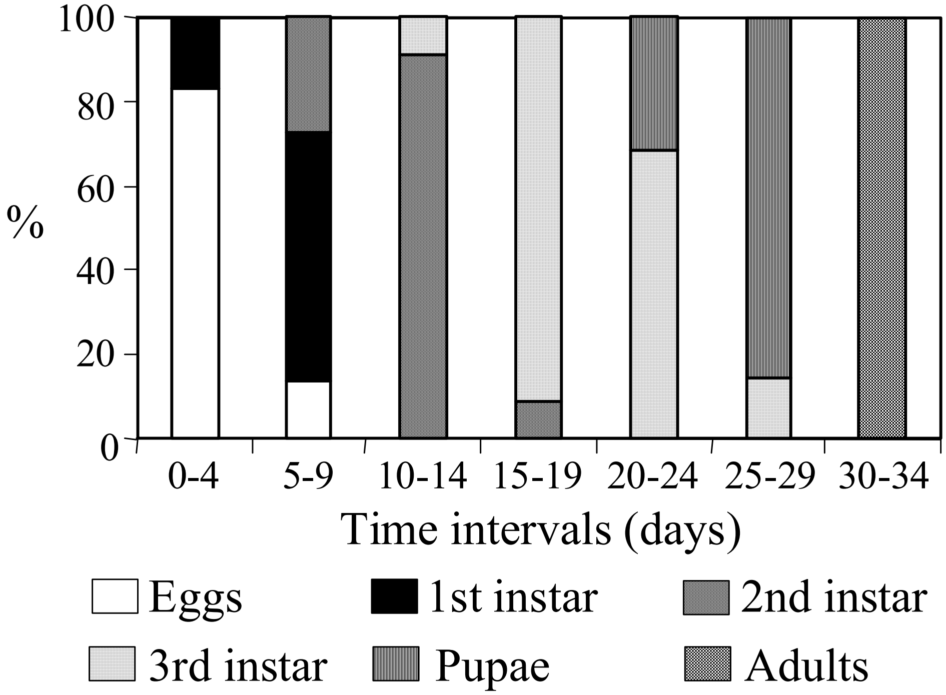

Fig. 2. The percentages of eggs, larval instars, pupae, and adults of *Alcidodes sedi* found in *Bryophyllum delagoense* stems dissected at various intervals after plants had been exposed to adults for 48 h.

Fully developed adults were found in dissected stems 31 days after adult exposure (Fig. 2). Adults had a mean (±SE) mass of 0.04 ± 0.00 g (range = 0.02-0.06 g), mean (±SE) length of 7.9 ± 0.06 mm (range = 6.3 – 9.1 mm; *n* = 81), and a mean (±SE) maximum pronotal width of 3.0 ± 0.02 mm (range = 2.5–3.5 mm;

n = 81). Adults were dark brown to grayish brown, with large whitish patches on the posterior corners of the pronotum, two broad, irregular, yellowish patches across each elytron, and a V-shaped whitish mark near the elytral apex. Each femur was adorned with a sharp ventral tooth, those of the front legs much larger and denticulate on their distal edges. They were rather cumbersome and did not take flight when disturbed, but generally rather dropped to the ground and played dead.

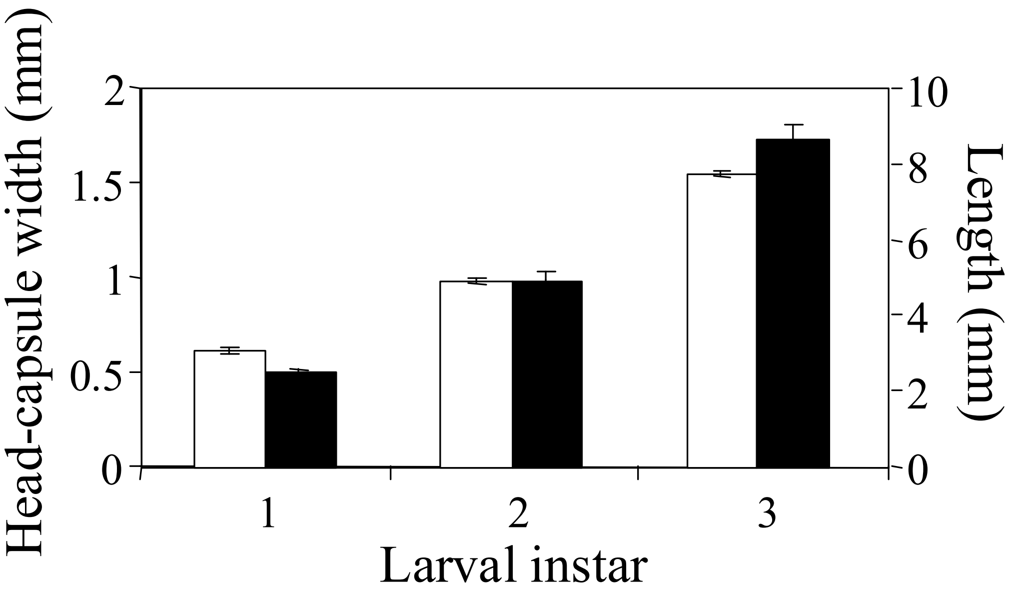

Fig. 3. The head-capsule widths (clear bars) and body lengths (dark bars) of *Alcidodes sedi* larval instars, with standard error bars.

3.2.2. Feeding damage.

There were no significant differences in stem diameter, stem length, and number of leaves between control and experimental plants prior to the start of the experiment (Table 2). Ten days of adult and early instar larval feeding did not significantly reduce stem diameter, but caused a significant decrease in the stem length and number of leaves on experimental plants (Table 2). After 30 days, control plants were significantly taller and had more leaves than experimental plants (Table 2). Control plants were also significantly heavier at the termination of the trial, with a mean (±SD) wet weight of 70.3 ± 18.2 g (*n* = 10), compared to experimental plants (35.6 ± 6.1 g (*n* = 5) (df = 13; *t* = -5.43; *P* < 0.001).

3.2.3. Dispersal ability.

None of the 20 adults that had been in a container with *B. delagoense* plants flew off the hotplate. Of the 10 starved adults placed on the hotplate after seven days, none took flight, while five of those starved for 10 days readily flew off the plate. Although no *A. sedi* adults were dissected to determine the status of their flight muscles, studies of other weevil species have found that in situations where food resources are absent for a number of days, adults have the ability to re-allocate resources from reproduction to flight-muscle development (Buckingham and Passoa, 1985). We speculate that this may also occur in *A. sedi* adults.

3.2.4. Survival and longevity.

Adults (*n* = 10) without access to *B. delagoense* or moisture died within 25.1 ± 4.09 (mean ± SE) days; of these, nine adults survived for 16-26 days, and a single adult survived for 61 days. All of the 20 adults on *B. delagoense* bouquets were still alive after more than 239 days, when the trial was terminated.

3.3. Host Specificity

Alcidodes sedi could complete its development on all of the *Bryophyllum* species tested, with the exceptions of *B. gastonis-bonnieri* and *B. fedtschenkoi* (Table 3). Development also occurred on *Kalanchoe sexangularis*, *K. rotundifolia* (Haw.) Haw., *K. blossfeldiana* (von Poellnitz) Boiteau, *C. orbiculata*, and *Echeveria* sp. hybrid. There was no development on *K. beharensis*, any of the *Crassula* species, *Portulacaria afra* (Portulacaceae), or *Senecio barbertonicus* (Asteraceae) (Table 3).

Table 2.

Mean stem diameters, stem lengths, and number of leaves of plants exposed to a pair of *Alcidodes sedi* adults and early instar feeding for 10 days, and subsequent larval feeding for 20 days (Expt.), compared to 10 control (Control) plants

Days	Stem diameter (mm)		Stem length (mm)		Number of leaves	
	Control ($n = 10$)	Expt. ($n = 5$)	Control ($n = 10$)	Expt. ($n = 5$)	Control ($n = 10$)	Expt. ($n = 5$)
0	6.1 (0.2) a	5.9 (0.2) a	426 (13.9) a	443 (11.1) a	62.2 (3.2) a	65.4 (3.7) a
10	5.7 (2.0) a	5.7 (0.2) a	501 (14.2) a	475 (18.0) b	75.6 (14.2) a	48.6 (6.2) b
30	5.9 (0.2) a	5.7 (0.3) a	547 (44.3) a	463 (26.9) b	84.9 (15.8) a	38.8 (6.6) b

Figures in parenthesis represent standard errors.

Pairs of means in the same row followed by the same letter are not significantly different (Student's *t*-test; $P > 0.05$).

4. Discussion

Alcidodes sedi has extended its host range to include *B. delagoense* and could be limiting the spread of this introduced plant in South Africa. The weevil is easy to rear, oligophagous, and damaging. These factors make it a potential candidate for the biological control of *B. delagoense* in Australia, provided that approval can be obtained for its release. Preliminary host range trials have indicated that *A. sedi* can complete its development on a number of other species in the Crassulaceae, many of which have been introduced to Australia as ornamentals. It is unlikely that *A. sedi* will develop on any of the eight native Australian *Crassula* species, these being the only native Crassulaceae, in Australia because no development occurred on any of the four South African *Crassula* species tested. Also, most of the native Australian species all have stem diameters of less than 3 mm (M. Hannan-Jones, Queensland Department of Natural Resources & Mines, Australia, personal communication) and are probably too narrow to support the development of *A. sedi* larvae. *Osphilia tenuipes* had a similar host range to *A. sedi* in preliminary trials in South Africa (Witt, 2004) and failed to develop on any of the native *Crassula* spp. Tested in Australia (W.A. Palmer, Queensland Department of Natural Resources & Mines, Australia, personal communication) despite being much smaller than *A. sedi*. Final instar *A. sedi* and *O. tenuipes* larvae had mean head capsule widths (± SE) of 1.6 ± 0.02 mm (this paper) and 1.0 ± 0.009 mm (Witt, 2004), respectively.

Table 3.

Host range of *Alcidodes sedi* as determined by no-choice trials

Test plants	*n*[b]	Number of adults emerged (mean ± SE)	Number of days to emergence (mean ± SE)	Damage [c]
Crassulaceae				
Bryophyllum delagoense[a]	12	12±2.4	77.3±1.1	S
B. proliferum[a]	3	26±6.2	76.5±1.3	M
B. pinnatum[a]	3	16±3.1	66.2±0.7	S
B. daigremontianum[a]	3	3.3±0.7	98.3±3.0	M
B. fedtschenkoi[a]	3	0	-	Neg.-M
B. gastonis-bonnieri[a]	3	0	-	Neg.-M
Kalanchoe sexangularis	3	8.7±4.3	96.1±2.1	M
K. beharensis Drake[a]	3	0	-	Neg.
K. rotundifolia (Haw.) Haw.	3	1±1	85.3±2.4	Neg.-M
K. blossfeldiana (von Poellnitz) Boiteau[a]	3	3.3±0.9	68.6±1.7	S
Cotyledon orbiculata	3	5±2.6	76.7±1.8	M
Crassula sarmentosa Harv.	3	0	-	M
C. swaziensis Schonl.	3	0	-	Neg.
C. rupestris Thunb.	3	0	-	Neg.
C. ovate	3	0	-	Neg.
Echeveria sp. Hybrid	3	1.3±0.7	65±5.2	S
Portulacaceae				
Portulacaria afra Jacq.	3	0	-	N
Asteraceae				
Senecio barbertonicus Klatt	3	0	-	N

[a] Plant species native to Madagascar but naturalized in southern Africa.

[b] Number of replicates of plants.

[c] Estimated damage caused by adult and larval feeding (N, none; Neg., negligible; M, moderate; S, substantial).

Alcidodes sedi was first collected and reared from "stems of a *Sedum* species" (Crassulaceae), in Pretoria, South Africa, in 1937, and was described the following year (Marshall, 1938). It was initially assumed that *A. sedi* was an accidental introduction to southern Africa since *Sedum* species are native to northern temperate and subtropical regions (Webb et al., 1988). However, Alonso-Zarazaga and Lyal (1999) did not list the genus *Alcidodes* as occurring in Europe. Further investigations also revealed that many native South African *Crassula* species were previously incorrectly placed in the genus *Sedum* (Tölken, 1985). The possibility therefore exists that *A. sedi* was collected off a species now placed in the genus *Crassula*. This however seems unlikely considering that none of the four *Crassula* species tested supported development of *A. sedi* and no weevils were recovered on any of the three *Crassula* species surveyed in the field in South Africa. In any event, misidentification of the original host plant seems to be a distinct possibility as many species within the Crassulaceae show high intraspecific variation (Tölken, 1985). It is also highly unlikely that *A. sedi* is native to Madagascar as four intensive surveys of *B. delagoense* in southern Madagascar did not reveal any *A. sedi* larvae, adults, or *A. sedi*-type damage (Witt and Rajaonarison, 2004). There were also no specimens of *A. sedi* in the insect collection housed at the Tsimbazaza Botanical Gardens in Antananarivo, Madagascar and no specimens held in other collections were collected anywhere else other than in southern Africa.

The phenomenon of native insects colonizing introduced plants is well known and should not prevent the selection of *A. sedi* as a potential biocontrol agent. Many preadapted insects found on native thistles in southern California have colonized the introduced thistle *Cirsium vulgare* (Savi) Tenore (Goeden and Ricker, 1986), and insects on native *Senecio* species in Australia have also been found on the introduced weed *Senecio madagascariensis* Poir. (S. Neser, ARC-PPRI, Pretoria, South Africa, personal communication). The ability of insect herbivores to colonize introduced plants depends, to a large extent, on the taxonomic and biochemical relatedness of the introduced plants to native plants (Strong et al., 1984). Connor et al. (1980) demonstrated that native and introduced trees that are chemically and taxonomically unique have impoverished insect herbivore faunas, compared to those that have several close relatives occurring sympatrically. This appears to be the situation in southern Africa, where there are approximately 240 species in the Crassulaceae, comprising five genera (Dreyer and Makwarela, 2000), and which harbor a diversity of insect species (Kroon, 1999; A.B.R. Witt, unpublished). Some of these insects are preadapted to colonize other closely related introduced plants such as *Bryophyllum* species. In contrast, Australia has only eight indigenous Crassulaceae, all *Crassula* species (Tölken, 1981), with the result that the pool of potential insect colonizers is substantially smaller than in South Africa (M. Hannan-Jones, Queensland Department of Natural Resources & Mines, Australia, personal communication). More than eight native insect species are known to complete their development on *B. delagoense* in South Africa (A.B.R. Witt, personal observation), while only some thrips species and the introduced *Aphis sedi* Kaltenbach (Hemiptera; Aphididae) are reported to have colonized the plant in Australia (M. Hannan-Jones, Queensland Department of Natural Resources & Mines, Australia, personal communication).

According to Hokkanen and Pimentel (1989), the probability of biocontrol success in using an insect agent that originates from a congeneric host plant and that is not naturally associated with the target plant would be approximately 75% greater than with the so-called 'old-association' approach, in which the agent and target plant have a long co-evolved association. This is based on the lack of co-evolved homeostasis, in which the plant is poorly adapted to cope with herbivory from the new insect associate. This would be unlikely in this case because *O. tenuipes*, collected from *B. delagoense* in its native range in Madagascar (Witt, 2004), occupies the same plant part as *A. sedi* in southern Africa. This so-called 'new-association' approach would only be successful as promulgated by Hokkanen and Pimentel (1989) if the new associate attacked plant tissues that were not normally attacked in the native range of the plant. It is likely that *B. delagoense* has evolved defenses to deal with weevil stem-boring, which would not necessarily make *A. sedi* a more effective biological control agent than *O. tenuipes*.

Osphilia tenuipes is a better option for biocontrol of *B. delagoense* in Australia because of its ability to disperse readily and its high reproductive rate (Witt, 2004). *Alcidodes sedi* should, however, not be discarded as many potential biocontrol agents fail to establish when released (Julien and Griffiths, 1998), and should this be the case with *O. tenuipes*, then *A. sedi* could possibly be utilized. Additional host range trials will have to be undertaken in Australia, on native and introduced species in the Crassulaceae, before *A. sedi* can be considered for release. The fact that it can complete its development on many native southern African species in the Crassulaceae, some of which have been introduced to Australia as ornamentals, may be problematic. However, development on *B. proliferum* and *B. pinnatum* could be beneficial as both species are becoming increasingly invasive in Australia (Hannan-Jones and Playford, 2002).

Although *B. delagoense* is listed as high priority invader in South Africa, it does not appear to have fulfilled its invasive potential and has a fairly limited distribution (Henderson, 2001), despite having been naturalized for some 170 years. This situation may have arisen because of damage inflicted by *A. sedi* and other newly associated insect natural enemies of crassulaceous plants. However, should the weed status of *B. delagoense* in South Africa increase dramatically in the future, mass-rearing of *A. sedi* coupled with augmentative releases at isolated infestations may provide an alternative to mechanical and chemical control. Indeed, South Africa has few biocontrol options for *B. delagoense* because of the risk of nontarget attacks on native Crassulaceae by imported Madagascan insects like *O. tenuipes* (Witt, 2004).

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CHAPTER 9

THE INFLUENCE OF SOIL TYPES AND COMPETITION ON THE MADAGASCAN ENDEMIC *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE) IN SOUTH AFRICA, AN INVASIVE SPECIES IN AUSTRALIA.

Justification

According to Catford *et al*. (2009) the main factor that affects invasion success is propagule pressure, which is based on the size and frequency of introductions; abiotic characteristics, which incorporate ecosystem invasibility based on physical conditions; and biotic characteristics which include the characteristics of invading species, the recipient community and their interactions. In the previous chapters I looked at some of the biotic characteristics and provided evidence that *B. delagoense* does not require specialized pollinators, and may in fact be autogamous, and hypothesized that biotic interactions such as enemy release (Blumenthal, 2006; Keane and Crawley, 2002) could be facilitating the invasion of *B. delagoense* in Australia. Introduced populations are exposed to fewer species of pathogens and insect herbivores (Colautti *et al*., 2004; Mitchell *et al*., 2006). However, invaders with native congeners may be more likely to acquire new enemies from those congeners (Mack, 1996) which we have demonstrated to be the case for *B. delagoense* in South Africa, but not Australia where there are no native species in the genus *Kalanchoe* with one possible exception. There is some dispute as to the origin of *K. crenata* in Australia (see Kenneally 1983). Here, we regard it as being introduced. Additional biotic characteristics such as the competitive ability of the introduced species and competition from other species in the recipient environment can also prevent an introduced species from becoming invasive (Catford *et al*., 2009).

Competition from the recipient community is a principal mechanism for biotic resistance to invasion (Emery and Gross, 2006) but can be overcome depending on the competitive ability of the introduced species and/or its ability to take advantage of disturbance events, such as grazing or fire which may reduce competition from native plants (Elton, 1958; Rejmánek and Richardson, 1996). Weak competitors will only be able to establish and spread if they can benefit from disturbance events and if they can tolerate the climatic and edaphic conditions available in the disturbed environments. Abiotic or environmental characteristics of a habitat have long been regarded as a fundamental determinant of the potential distribution of introduced plant populations (Mitchell *et al.*, 2006). Introduced species that do not have specific soil or mineral requirements should have the ability to establish and spread on a wide range of soil types within their introduced ranges (Huenneke *et al.*, 1990). The fact that *B. delagoense* is invasive in Australia and not South Africa, despite a much longer residence time in southern Africa, suggests that factors other than species traits, but rather characteristics of the recipient environment, or a combination of both, or even genetic differences amongst the populations, may explain its invasiveness in Australia. For example, there are two *Parthenium hysterophorus* L. biotypes in Australia; one is relatively benign while the other is very aggressive (Navie *et al.*, 1996); we may have a benign or less aggressive biotype of *B. delagoense* in southern Africa. Interactions between propagule pressure, abiotic characteristics and biotic characteristics affect invasion outcomes and should be central to the way invasion is viewed (Catford *et al.*, 2009). In this chapter we investigate the competitive ability of *B. delagoense* and its ability to grow in a wide range of soil types differing in soil particle-size distribution and elemental composition. The results are used to explain which factors may be responsible for the contrasting situation with regard to the invasiveness of *B. delagoense* in South Africa and Australia, a contribution which should provide us with a better understanding of the factors responsible for the invasion success of many introduced plant species.

These laboratory trials indicated that *B. delagoense* has the ability to grow on a range of soil types. This capacity to tolerate sand and clay soils, varying in nutrient levels is a beneficial plant species trait (Huenneke *et al.*, 1990) which will enable *B. delagoense* to establish and spread in a wide variety of habitats throughout the world provided that other factors essential for its growth and reproduction are met, including reduced competition from native species in its country of introduction (Catford *et al*., 2009). *Bryophyllum delagoense* growth was influenced by competition from a grass species but less so in clay soils when water was not limiting. Where competition from other species is low, the invasion success of *B. delagoense* can be attributed to its ability to tolerate a wide range of soil types. Disturbance events are known to increase resource availability, contributing to the success of invasive species (Elton, 1958; Rejmánek and Richardson, 1996) provided that the introduced plant has sufficient phenotypic plasticity to deal with the available edaphic conditions (Mitchell *et al*., 2006). Other factors which may contribute to the invasion success of *B. delagoense* are considered in subsequent chapters.

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The influence of soil types and competition on the Madagascan endemic *Bryophyllum delagoense* (Crassulaceae) in South Africa, an invasive species in Australia.

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Abstract

The biological attributes of species and habitat compatibility are the main determinants of invasion success or failure. Other factors such as initial inoculum size, residence time, propagule pressure, and chance events may also determine whether a species will invade or not. In this study we only considered the influence of soil type and competition on the growth of *Bryophyllum delagoense*, a Madagascan endemic, which is present in South Africa but invasive in Australia. We compared the particle-size distribution, pH and elemental composition of soil samples collected from seven *B. delagoense* sites, at five field localities in southern Africa. The growth of plants in these soil types and perlite were compared under laboratory conditions. There were no significant differences in plant wet weights and number of leaves from plants grown in the different treatments, although there were some significant differences in the above- and below-ground lengths of plants. Plants grown in higher nutrient soils were generally larger than those grown in other soils at the termination of the trial but this was possibly confounded by the cation exchange capacity (CEC) of the various soils. Plants grown in perlite had significantly longer roots than those grown in soils which suggest phenotypic plasticity in a nutrient-limited growth medium. Competition trials indicated that the water-holding capacity of two different soil types, and competition from a grass, *Eragrostis tef*, influenced the growth of *B. delagoense*. Where competition from other species is low, the invasion success of *B. delagoense* can be attributed to its ability to tolerate a wide range of soil types. We conclude that reduced competition from native species as a result of disturbance events such as overgrazing may be facilitating the invasion success of *B. delagoense* in Australia.

Key words: Bryophyllum delagoense • competition • invasion potential • shade •soils

Introduction

The Crassulaceae is a medium-sized family of leafy succulents with an estimated 1500 species (Eggli et al. 1995), which predominantly occur in semi-arid to arid and rocky or mountainous habitats of the temperate and subtropical zones of the northern hemisphere and southern Africa ('t Hart and Eggli 1995). While the family does not include any crop plants, a considerable number of species are important in the horticultural industry and as such have been introduced as ornamentals to many countries worldwide (Eggli et al. 1995). A large number of these introduced species have become invasive, particularly those in the genus *Bryophyllum*, which is endemic to Madagascar (Hannan-Jones and Playford 2002).

Why some introduced plants become invasive can be attributed to either characteristics of the invader itself or the habitat which is invaded (Elton 1958; Rejmánek and Richardson 1996; Colautti et al. 2004; Levine et al. 2004; Catford et al. 2009). Other factors such as the number of plants initially introduced, residence time, and propagule pressure also need to be considered (Rejmánek et al. 2005). Features of the introduced plant associated with its sexual reproductive potential, ability to propagate vegetatively and disperse, are important correlates of invasiveness (Richardson and Cowling 1992; Crawley et al. 1996). However, many introduced species which possess these traits fail to become invasive due to the presence of multiple barriers (Richardson et al. 1992; Mack 1996) which include losses caused by generalist or pre-adapted natural enemies (Witt, McConnachie and Stals submitted), absence of pollinators, and competition (Crawley 1987; Cronk and Fuller 1995; Richardson et al. 2000; Colautti et al., 2004; Witt, Nongogo and Byrne submitted), while climatic incompatibility and edaphic qualities may also serve as barriers (Mitchell et al. 2006).

This paper investigates some of the factors which may contribute to the invasive potential of *Bryophyllum delagoense* (Ecklon and Zeyher) Schinz

(Crassulaceae; mother-of-millions, finger plant), which is naturalized in many countries worldwide and is particularly invasive in Australia (Hannan-Jones and Playford 2002) and a declared weed in South Africa (Henderson 2001), although it is not considered to be particularly invasive anywhere on the African mainland. We considered some of the habitat requirements of *B. delagoense* by analysing a selection of soil types on which it occurs in South Africa, to ascertain if *B. delagoense* has specific requirements in terms of soil particle-size distribution and elemental composition. We also compared its growth in these soil types and a control substrate. Introduced species that do not have specific soil or mineral requirements would have the ability to establish and spread on a wide range of soil types within their introduced ranges (Huenneke et al. 1990). The competitive ability of *B. delagoense* on different soils and under different watering regimes was also assessed because efficient competitors for limiting resources are likely to be successful invaders (Rejmánek et al. 2005). We consider the overall findings of this study in an attempt to explain why *B. delagoense* is so invasive in Australia but not in southern Africa.

Materials and Methods

Soil types

To assess soil characteristics of invaded sites and determine if *B. delagoense* is tolerant of a wide range of soil types, soil samples were collected from seven *B. delagoense* sites, at five field localities in southern Africa (see Table 1). Soils were collected over a broad geographical range and soil composition was chosen rather than a representative sample from every site, to gain a better understanding of how the plant copes with a range of soils. Particle-size distribution (percentage), pH and elemental composition (total Na, K, Ca, Mg) in milliequivalents (me) per 100 g of soil were measured. Soil samples were analyzed using the Agricultural Research Council – Institute for Soil, Climate and Water (ARC-ISCW) laboratories in Pretoria according to methods described by the Non-affiliated Soil Analysis Working Committee (1991). The growth of *B. delagoense* plantlets in each of these soils and perlite was measured. Perlite was used as a control because field observations indicated that in some cases, *B. delagoense* has the ability to grow in places where there is no visible soil – for the purposes of this trial perlite was considered to be a medium containing little to no nutrients (www.perlite.org). Each of the seven soils and perlite were randomly assigned to eight plug positions in 10 seedling trays. Plantlets, which grow on the apex of the leaves and establish by dropping to the ground, were collected from mature nursery plants and mixed together. These were then placed singly on each soil treatment and watered with 25 ml of tap water, which is known to contain low levels of nutrients, every 2-3 days (n=10/treatment). Trays were placed in growth chambers (30°C day; 20°C night: photoperiod 12L:12D) and randomly repositioned in the chamber daily. The trial commenced in December 2006 and was terminated in March 2007, whereupon the length (above- and below-ground), wet weight (above- and below-ground) in grams to four decimal places (Mettler AL54/00 Analytical Balance) and number of leaves of each plant on each soil type and perlite was recorded to ascertain if there were differences in growth between the treatments.

Analysis of variance (ANOVA) was used to test for differences in length, wet weight and number of leaves of plantlets in the different treatments. Data were analysed using GenStat for Windows (Anonymous 2003). The data were acceptably normal with heterogenous treatment variances. Treatment means were separated using Fishers' protected t-test least significant differences (LSD) at the 1% level of significance (Snedecor and Cochran 1980). The same analysis was used to test for differences in the shoot:root ratios of plantlets grown in the various soil types and perlite.

Competition

The growth of *B. delagoense* in the presence of another species was assessed by growing plantlets in containers together with the indigenous east African grass, *Eragrostis tef* (Zucc.) Trotter (Poaceae) (RF 7400; Prime ID: 07ED; Cultivar New Red; source: ARC-Range and Forage Institute, South Africa). *Eragrostis tef* was used because viable seeds were readily available and germination success was assured. Soils for these trials were collected from two different localities near Roodeplaat Dam, Pretoria, South Africa and analysed for their soil particle distribution and pH. One of the soil types was dominated by clay (35.6% sand; 11% silt; 49.6% clay; pH=6.5) and the other by sand (97% sand; 1.1% silt; 0.1% clay; pH=8.07). The clay was placed in 20 pots (30cm diameter), and sand in another 20. Ten plantlets were randomly placed in each of the 40 pots. One gram (c. 3242) of *E. tef* seeds were randomly sown in each of 10 pots containing clay and 10 pots with sand. No grass seeds were sown in the remaining 20 containers. Two watering regimes were used; half of each treatment received 100ml of water 2-3 times per week and the other half received 300ml at the same time. Plantlets were planted in January 2005 and plant height recorded in November 2005.

The experiment was designed as a randomised complete block design, with five replicates for each treatment. Analysis of variance was used to test for differences in the size of *B. delagoense* plantlets, in sand and clay soils, with or without grass as a competitor and watering of 100ml versus 300 ml. The data were acceptably normal with heterogeneous treatment variances. Treatment means were separated using Fisher's protected t-test with least significant difference (LSD) at the 5% level (Snedecor and Cochran 1980), and the *F*-probability from the ANOVA taken as significant at 5%. Data were analysed using the statistical programme GenStat for Windows (Anonymous 2003).

Results

Soil types

Soils collected from *B. delagoense* field infestations were predominantly sandy with very little silt or clay (Table 1). However, there was considerable variation in particle size distribution of the sandy component of the soils from the various localities. Soils from the Sabie locality, site A, were dominated by coarse sand (45.4%), while those from Mkhuze were dominated by fine sand (67.5%). There was a higher percentage of silt (19.9%), fine silt (8.3%), and clay (14.6%) in the soil from the Addo site than elsewhere. Soil pH ranged from 6.23 (slightly acidic) to 7.96 (moderately alkaline) with the percentage of carbon ranging from 0.42 to

3.09% (Table 2). Soils collected from Mkhuze had the lowest levels of carbon, Na, Ca, and Mg while those from Sabie Site B had the highest or second highest levels of these elements including K and could be considered the most nutrient-rich soils of all soils tested.

Table 1 Particle size distribution (%) of the soils collected from five field localities (seven sites) on which *Bryophyllum delagoense* was recorded in South Africa

Texture fraction	Mkhuze	Addo	False Bay	Sabie Site A	Sabie Site B	Eiland Site A	Eiland Site B
Coarse sand (< 2 mm)	0.8	3.3	21.1	45.4	22.3	18.5	11.5
Medium sand (< 0.5 mm)	24.3	8.8	54.4	17.4	21.7	22.9	23.6
Fine sand (< 0.25 mm)	67.5	24.8	10.0	14.5	24.6	27.2	27.1
Very fine sand (< 0.106 mm)	2.3	18.8	2.8	6.5	10.4	10.5	11.5
Silt (< 0.05 mm)	0.9	19.9	2.0	4.7	6	6.7	13.2
Fine silt (< 0.02 mm)	0.7	8.3	2.3	4.5	5.4	5.5	6.3
Clay (< 0.002 mm)	1.8	14.6	5.9	4.9	7.8	6.7	4.7

At the termination of the trial plantlets grown in soils collected from Mkhuze had shorter above and below ground length's, fewer leaves, and lower above ground wet weights than any of the plants grown in the other soils (Table 3). Plantlets grown in soils collected from Sabie Site B performed better, on average, than any of the plantlets from the other treatments. In the case of Mkhuze and Sabie Site B there appears to be a relationship between the levels of macro-nutrients, pH, percentage carbon and growth rates (see Table 2). However, despite the apparent influence of soil macro-nutrients, pH and percentage carbon on plant growth rates, plantlets grown in perlite were larger than many of the plants grown in soils. In fact, the roots of plantlets grown in perlite were significantly longer than those grown in other treatments, except False Bay soils.

Table 2. Percentage carbon, pH and levels of various elements in milliequivalents/100grams (me/100g) in the soils collected from five field localities (seven sites) on which *Bryophyllum delagoense* was recorded in South Africa

	Mkhuze	Addo	False Bay	Sabie Site A	Sabie Site B	Eiland Site A	Eiland Site B
% Carbon	0.42	0.85	3.09	1.63	2.73	1.19	0.93
pH	6.23	7.56	7.93	6.47	7.96	6.74	7.50
Na	0.11	0.18	0.51	0.064	1.718	0.612	0.294
K	0.27	0.42	0.22	0.198	0.294	0.282	0.221
Ca	1.47	7.48	16.34	2.661	7.840	4.306	3.025
Mg	0.82	2.03	1.67	1.097	1.741	1.103	1.023

Table 3. Mean (± SE) growth parameters of plants grown in soils collected from five field localities (seven sites) and perlite.

Localities	N	Above ground length (cm)	Below ground length (cm)	Above ground wet weight (g)	Below ground wet weight (g)	Number of leaves
Perlite	10	6.41±0.77 ab	4.41±0.96 c	0.68±0.15 a	0.0285±0.01 a	10.10±1.06 a
Addo	10	7.1±1.14 b	2.4±0.36 ab	0.80±0.22 a	0.0259±0.01 a	10.60±1.59 a
Eiland Site A	10	5.7±0.76 ab	2.2±0.37 ab	0.30±0.06 a	0.0227±0.01 a	8.00±1.04 a
Eiland Site B	10	6.7±0.57 b	2.4±0.30 ab	0.43±0.09 a	0.0283±0.01 a	8.80±0.59 a
False Bay	10	5.3±0.76 ab	2.9±0.30 bc	0.56±0.16 a	0.0310±0.01 a	9.80±1.21 a
Mkhuze	10	3.7±1.17 a	1.1±0.29 a	0.28±0.13 a	0.0349±0.02 a	7.10±1.90 a
Sabie Site A	10	6.4±1.06 ab	2.0±0.25 ab	0.46±0.13 a	0.0291±0.01 a	9.30±1.15 a
Sabie Site B	10	7.9±0.82 b	2.9±0.26 b	0.87±0.25 a	0.0279±0.01 a	10.60±1.16 a
F-probability		0.008	< 0.001	0.021	0.991	0.206

[1] Values with the same letters within a column are not significantly different ($p < 0.01$; Fisher's protected *t*-test LSD).

Competition

Soil types in terms of water-holding capacities and competition influenced the growth of *B. delagoense* (Figure 1). On clay soils, which retain water, *B. delagoense* grew significantly taller under the high watering regime, and significantly tallest when *E. tef* was absent. However, low and high water treatments caused no significant size difference in *B. delagoense* plants growing in sand, with or without *E. tef*, which can be assumed to be as a result of sandy soils draining rapidly, rather than differences in nutrients.

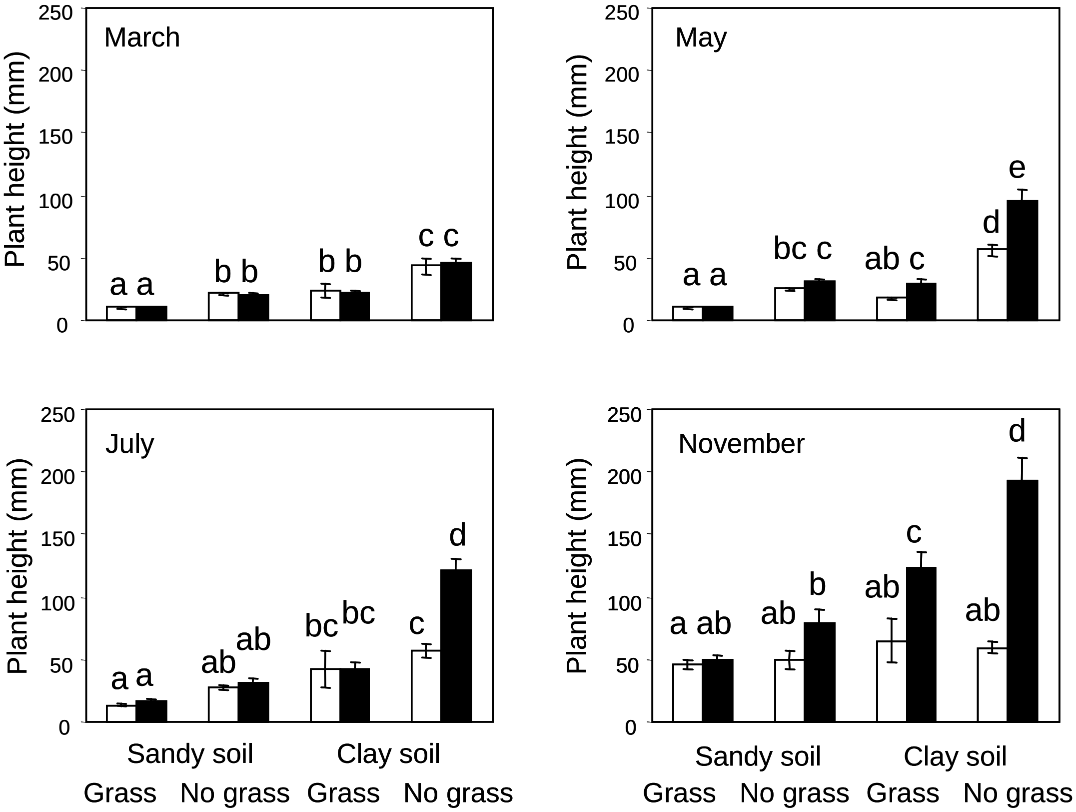

Figure 1. The mean (± SE) height of *Bryophyllum delagoense* plantlets grown with and without *Eragrostis tef* in different soil types and under different watering regimes (100 ml = clear bars; 300 ml = solid bars). Bars with the same letters are not significantly different ($p < 0.01$; Fisher's protected t-test LSD).

Discussion

One of the factors preventing plant species from establishing in novel environments could be the absence of suitable soil types. However, based on the current distribution of *B. delagoense* and the results of this study it would appear that this invasive plant species can tolerate a wide range of soils. In its native range in Madagascar, *B. delagoense* grows on igneous and metamorphic basement rocks, which are mainly granites and migmatites, and also on unconsolidated sands, mainly between the towns of Beloha and Tranoroa (Witt and Rajaonarison 2004). In Queensland, Australia, *B. delagoense* is found on dry skeletal soils or rock faces, shallow stony soils in open forest or loose sand near the seashore (Hannan-Jones and Playford 2002). According to Batianoff and Franks (1998), *B. delagoense* has been collected on a range of soils in Australia, from sand to heavy clay, an indication that it does not have specific soil requirements in terms of particle size distribution. An analysis of soils in which *B. delagoense* was found growing in South Africa revealed that it occurs predominantly on soils dominated by sand with variable levels of macro-nutrients and organic carbon.

Plants reveal information about the properties of soils, particularly their nutritional status and pH which often has a direct effect on the viability of plants (Larcher 1983). In our study, *B. delagoense* grew faster on more alkaline soils with higher levels of organic matter. However, factors other than pH, organic carbon, and macro-nutrients may also have influenced growth rates. Although soils from Mkhuze had low nutrient levels they also had the lowest percentage of silt and clay which could also have influenced growth rates. According to Larcher (1983) soil physical properties are at least as important as soil chemistry in determining soil fertility. This is related to a soil's ability to hold cation nutrients at a specific pH and is commonly referred to as cation exchange capacity (CEC) (Seybold et al. 2005). Soil components known to contribute to CEC are clay and organic matter, and to a lesser extent, silt (Manrique et al. 1991). Sandy soils with very little organic matter have a low CEC which means that the amount of mineral nutrients available to plants is limited and result in the plants spending a considerable amount of energy "looking" for mineral nutrients (Seybold et al. 2005). The comparatively low levels of silt and clay (low CEC) and high levels of sand in Mkhuze soils, together with the low levels of nutrients may have affected growth rates. This was reflected in all of the growth parameters of plants grown in Mkhuze soils except for the below ground wet weights which were the highest of all of the treatments, although not significantly so. In this case plants may have re-allocated resources to root growth in order to enhance the plant's capacity to source nutrients. The ability of *B. delagoense* to allocate resources to root production in nutrient-limited environments demonstrates phenotypic plasticity, a trait which has long been considered as contributing to the success of weeds (Baker 1965).

Bryophyllum delagoense plantlets grown in perlite showed a similar growth pattern to those observed in plants grown in soils collected from Mkhuze, with significantly longer below ground lengths than in any of the other treatments (with the exception of those grown in soils collected at False Bay). Perlite is sterile and contains no nutrients but the addition of tap water would have introduced low levels of some minerals and macro-nutrients. Perlite has the ability to retain moisture with a water adsorption of 200-600% by weight (www.perlite.org). *Bryophyllum delagoense* has also been found growing as a pioneer on volcanic ash soils on Mt. Sakurajima, Japan, which contain negligible amounts of nitrogen, an essential nutrient for plant growth (Gang and Miyauchi 2000). This capacity to grow in areas with little to no soil is borne out by casual observations from other parts of the world, where naturalized plants have been seen growing in pavements and even on tile roofs (S. Neser, pers. comm.). Therefore low soil moisture, and not a lack of suitable soil types, may partly explain why *B. delagoense* was never recorded in the south-west of Madagascar, which receives less than 250 mm of rainfall per year, compared to the mean of 490 mm per annum within its recorded range (Sutherst and Maywald 1985). This potential to grow in a variety of soil types is a therefore a beneficial attribute which allows *B. delagoense* to establish in wide range of habitats. However, its competitive ability suggests that it will only be able to establish in new habitats if it can outcompete native species in the recipient environment or take advantage of episodic disturbance events.

The competition trials showed that *E. tef* had a negative impact on *B. delagoense* growth which was mediated through water availability, indicating that *B. delagoense* may establish and grow better in "enemy-free space" in areas where moisture is not limiting. In Australia, *B. delagoense* tends to spread along watercourses and areas where there is a "lack of competition from other plant species" (Queensland Regulatory Impact Statement, 1999). Support for the inability of *B. delagoense* to compete with other plant species can also be found from literature pertaining to the habitat requirements of other crassulaceous species (Thiede 1991; Uhl 1992; Jürgens 1995). This is also supported by Vilá et al. (2003) who found that succulents appeared to regenerate poorly in established vegetation where microsites may be limited. This can be attributed to the fact that many succulents have shallow root systems and therefore compete for limited resources in the upper soil layers, especially in more arid regions (Schenk and Jackson 2002; Shiponeni et al. 2007). *Bryophyllum delagoense* is therefore more likely to become established where disturbance events reduce the cover of adult plants, increasing space for colonization and reducing competition, especially between indigenous adult plants and *B. delagoense* juveniles (see Crawley et al. 1999).

Field evaluations, which examined the effect of grass cover on *B. delagoense* in South Africa, found fewer plants in quadrats with a high percentage grass cover (K Lorentz, unpubl. data). In Australia, *B. delagoense* is common in leaf litter or other debris, on shallow soils, in shady woodlands, and along roadsides and fence lines and on old rubbish dumps (Naughton and Bourke 2005). It also occurs frequently along creeks and rivers and spreads from these areas, especially after floods (Naughton and Bourke 2005). Riparian zones are susceptible to plant invasions due to hydrological disturbance from water level fluctuations and because water movement often facilitates the movement of propagules through the landscape (Fox and Fox 1986; Sauer 1988). Disturbance events such as floods may create refuges for inferior competitors (Bergelson et al. 1993) and move plantlets, as in the case of *B. delagoense*, to nutrient-rich microsites under fallen branches or logs (Hannan-Jones and Playford 2002). Introduced livestock and grazing management practices in Australia have exacerbated disturbance regimes and have had major negative impacts on the vegetation and soils of river banks (Wilson 1990; Walker 1993). According to Naughton and Bourke (2005), *B. delagoense* also tends to invade "run down" pastures far more readily than healthy pasture, confirming the weak competitive ability of *B. delagoense*. Overgrazed pastures therefore facilitate the invasion of *B. delagoense*, and with 58% of the total pasturage in Queensland in a degraded or deteriorating condition (Tothill and Gillies 1992), large areas may be more susceptible to invasion.

A study in an Andean grassland found that unpalatable or toxic species (*Bryophyllum delagoense* is highly toxic (McKenzie and Armstrong 1986)) and very low-growing species were significantly more abundant in heavily grazed sites compared to relatively protected sites (Adler and Morales 1999). In order to mitigate against the invasion of pastures in Queensland, Australia, landowners maintain strong pasture competition to suppress *B. delagoense* by planting, amongst others, exotic grasses such as *Panicum maximum* Jacq. and *Cenchrus ciliaris* L. (Hannan-Jones and Playford 2002). The introduction of exotic grasses is promoted by many sectors because native perennial grasses in Australia may suffer more damage from introduced ungulate grazing than that sustained by introduced grass species, resulting in a shift to higher abundance of invasive plant species (D'Antonia et al. 2000). African grasses have evolved under grazing pressure from ungulates which makes Australian grasslands dominated by these exotic species more resistant to grazing and hence invasion by other species. Whenever disturbance factors such as overgrazing affect a landscape, competition from native plants is reduced, thus increasing the opportunity for invasive species to establish and spread. Competition from other species in the country of introduction has been known to reduce the size, density, and impact of many introduced plant species (Theoharides and Dukes 2007). This said it should be noted that although grasses introduced to Australia may suppress the growth of other non-native species many of them have subsequently become invasive (Lonsdale 1994).

This study has indicated that *B. delagoense* has the ability to grow on soils with a variable elemental composition. This trait will contribute to its invasive potential and ability to invade a wide variety of habitats dominated by various soil and vegetation types. However, the competition trials, and literature on the growth habitats of most crassulaceous species indicate that *B. delagoense* will probably establish more easily in disturbed areas in the absence of competition, especially in areas where soil moisture is not limiting. Overgrazing reduces the competitive ability of Australian grasses, more so than in Africa where grasses are adapted to grazing pressure and as a result are less likely to be invaded. Despite the ability of *B. delagoense* to grow in soils with variable amounts of macro-nutrients, and even in perlite, it has not become invasive in southern Africa, but is particularly problematic in Australia, which is an indication that plant traits alone cannot always predict invasion potential but that the characteristics of the recipient environment should be considered as equally important.

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CHAPTER 10

SHADE TOLERANCE IN THE INTRODUCED PLANT *BRYOPHYLLUM DELAGOENSE* – FACT OR FANTASY?

Justification

The preceding chapters have considered some of the biological traits and characteristics of the recipient environment that contribute to the invasion potential of *B. delagoense*. *Bryophyllum delagoense* possesses many biological traits that contribute to its invasive potential including rapid growth rate; production of massive amounts of seeds and the ability to propagate vegetatively through the production of plantlets; it is considered to be allelopathic; and adapted to a wide range of environmental conditions including the ability to grow on a range of soil types, traits shared by a number of invasive plants (Elton, 1958; Rejmánek and Richardson, 1996). It is also a popular garden ornamental and as a result has probably been introduced repeatedly to particular areas over a long period of time and over a large area, which has contributed to its invasive potential. Propagule pressure is considered to be one of the most important factors contributing to the invasiveness of species (Catford *et al*., 2009; Colautti *et al*., 2006). Although it is not a particularly strong competitor (Witt, Nongogo and Byrne submitted) it has the ability to colonize disturbed areas.

Despite the fact that *B. delagoense* is frost sensitive (Hannan-Jones and Playford, 2002) it has invaded frost-prone areas in Australia and South Africa, by establishing and growing under frost-hardy tree canopies. The shrubs and trees may protect *B. delagoense* plants from frost but they can also increase soil moisture, reduce extreme temperature fluctuations, enhance soil nutrient levels, and reduce the probability of mechanical or herbivory damage (Facelli and Brock, 2000; Holmgren *et al*., 1997; Jarvel and O’Connor, 1999). However, in order to take advantage of these favourable environmental conditions and protection from frost plants need to contend with lower light levels under trees (McAlpine and

Jesson, 2007). The ability to grow under different light intensities could therefore be considered an important attribute of invasive plant species, facilitating establishment in a wide range of habitats (Lososová *et al*., 2007; Parendes and Jones, 2000). Shade tolerance is especially important for short weed species (Lososová *et al*., 2007), such as *B. delagoense*, which are often overtopped by larger plants. In this study I attempted to ascertain which factors limit *B. delagoense* to tree canopies at Addo, in the Eastern Cape Province, South Africa and if this plant exhibits phenotypic plasticity which contributes to the success of many invaders (Rejmánek *et al*., 2005; Roy, 1990).

Bryophyllum delagoense exhibited optimal partitioning (Chapin 1991; Grime *et al*., 1986), and in so doing optimized growth, by allocating resources from stems and leaves to root production in nutrient-poor soils (see also Witt, Nongogo and Byrne submitted) and from roots to leaf and/or stem production in light-limited environments. This ability to maintain growth rates under limited-light environments allows *B. delagoense* to invade frost-prone areas by establishing and growing under trees and shrubs. This information has contributed to the body of literature on invasion biology by providing additional evidence for phenotypic plasticity in invasive plant species.

Ayanda Nongogo, the technician at the time that the trials were undertaken, assisted with the setting-up and running of trials. The manuscript has been submitted to the journal "Biological Invasions".

Shade tolerance in the introduced plant *Bryophyllum delagoense* – fact or fantasy?

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Abstract

Traits common to plant species which may improve their invasive potential include the ability of an individual or population to maintain fitness over a range of environments and sufficient phenotypic plasticity to exploit new environments. *Bryophyllum delagoense*, a Madagascan endemic, which is present in South Africa, but invasive in Australia, occurs predominantly under *Acacia karroo* trees at one site near Addo in the Eastern Cape Province, South Africa and also under trees in frost-prone areas in Australia. We tested the effect of light intensity and soil mineral and nutrient content of soils associated with shaded environments on growth rates to explain this observed pattern and whether *B. delagoense* showed optimal partitioning by shifting resource allocation to leaf and stem production in light-limited environments, and to root production in nutrient-limited environments. Plantlets grown in soil collected from under *A. karroo* trees, containing significantly high levels of clay and macro-nutrients, grew significantly larger than those grown in soil collected from unshaded areas between the trees. Plants grown in the latter soils showed increased resource allocation from the stems and leaves, to the roots compared to those grown in soils from the shaded areas. Plants grown in a glasshouse under varying shade intensities were significantly taller than those grown in direct sunlight. The below-ground wet weights of plants grown under 50% shade cloth were lower than those grown under higher irradiance but not significantly so. Plants grown under the deepest shade had shorter roots, weighed less and had fewer leaves. All plants were still alive at the termination of the trial, even those in the deepest shade, which indicates high physiological shade tolerance. There is some resource re-allocation in *B. delagoense* in light-limited environments, from the roots to the stems, especially under dense shade, although this is not evident with respect to the number of leaves. In the field, higher nutrient levels in the shade may also compensate for lower light levels. *Bryophyllum delagoense*, which is frost sensitive, appears to optimize growth under moderate shade by shifting resource allocation to stem production, allowing it to maintain fitness in frost-prone environments by growing under tree canopies. This ability to tolerate moderate shade will allow *B. delagoense* to invade frost-prone areas provided that there are trees and shrubs for protection.

Key words: Bryophyllum delagoense, invasion potential, phenotypic plasticity, shade, soils

Introduction

Bryophyllum delagoense (Ecklon and Zeyher) Schinz (Crassulaceae; mother-of-millions, finger plant), a succulent native to Madagascar, is naturalized in many countries worldwide. It is particularly invasive in Australia (Hannan-Jones and Playford 2002) and although it is a declared weed in South Africa (Henderson 2001), it is not considered to be particularly invasive anywhere on the African mainland. Despite its presence in southern Africa for more than 170 years, particularly in gardens, only three large field infestations, all less than 1-2 ha. in extent, were found in South Africa during intensive countrywide surveys (Witt, McConnachie and Stals submitted.). This is despite the fact that *B. delagoense* can tolerate a range of soil types differing in particle-size distribution, pH, and elemental composition and does not require specialized pollinators and may in fact be autogamous (Witt, Nongogo and Byrne submitted). However, other factors such as the acquisition of pre-adapted natural enemies may be inhibiting its spread in South Africa (Witt, McConnachie and Stals submitted) or characteristics of the Australian environment may make that country more susceptible to invasion. *Bryophyllum delagoense* is not a particularly strong competitor (Witt, Nongogo and Byrne submitted), and its susceptibility to fire (Witt and Nongogo submitted), may facilitate its invasion in regions where the competitive ability of native species has been compromised by disturbance, such as overgrazing. Overgrazing may also reduce the amount of vegetation available to carry a fire.

Despite *B. delagoense* being frost intolerant it has managed to extend its distribution, in Australia, into frost prone areas by establishing populations under tree canopies (Hannan-Jones and Playford 2002). A similar phenomenon has been observed at the largest known infestation near Addo in the Eastern Cape Province of South Africa, where *B. delagoense* is confined to the sub-canopy of *Acacia karroo* Hayne (Fabaceae) trees and is absent from the unshaded areas between the trees. This is in contrast to the situation in Madagascar where *B. delagoense* was mainly found on granite outcrops, in direct sunlight (Witt and Rajaonarison 2004). Trees reduce sub-canopy solar radiation (Belsky et al. 1989; Georgiadis 1989) which can have a dramatic influence on plant growth, survival and competitive interactions in the community (Valladares 2003). However, plants may possess sufficient phenotypic plasticity to optimize growth in a variety of environments by shifting resource allocation to leaf and stem production in light-limited environments, and to root production in nutrient- or water-limited environments (Bloom et al. 1985; Tilman 1988).

A species is said to be plastic when grown under different environmental conditions it exhibits differences in morphological or physiological traits, such as leaf characteristics (Ryser and Eek, 2000), photosynthesis (Thompson et al., 1992), and overall patterns of biomass allocation (Grime et al., 1986; Chapin 1991)). Plasticity has long been considered a trait contributing to the success of weeds (Baker 1974; Roy 1990). The ability to grow under different light intensities could therefore be considered as an important attribute of invasive plant species such as *B. delagoense*, enabling establishment in a wider range of habitats (Parendes and Jones 2000; Lososová et al. 2007). While there are presumably costs associated with being plastic, this ability may be another way invasive species can gain an advantage over indigenous species (Williams et al. 1995). One of the main factors affecting seedling plasticity and growth is likely to be light availability (Poorter 2001; Soltan 2003), although soil moisture (Fitter 1994), nutrients (Müller et al. 2000), herbivory (Schierenbeck et al. 1994), and competition (Grime 1979) can also be important. In the African savanna, trees can increase soil fertility (Belsky et al. 1989) and in so doing facilitate understory herbaceous growth, and in fact influence the overall species composition (Vetaas 1992). Soil fertility was found to compensate for lower light levels, with respect to flower number and flower weight in *Microstegium vimineum* (Trin.) A. Camus (Poaceae) (Claridge and Franklin 2002). Shade under trees and shrubs can also improve water use efficiency due to lower sub-canopy temperatures (Durr and Rangel 2003) and provide protection for plants against livestock grazing and trampling (Bruno et al. 2003).

In this study we attempt to ascertain if *B. delagoense* exhibits plasticity in leaf traits and biomass allocation under different light regimes which may explain its observed tolerance of a wide range of environmental conditions. To test, this we looked at the differences in soil particle size and nutrients between soils from under and between *A. karroo* trees, the growth rates of plantlets grown in these different soils and the influence of shade on resource allocation.

Materials and Methods

Study site

The study site (33°24’34”S; 25°42’57”E) was located north of Addo and east of Kirkwood in the Eastern Cape Province, South Africa. The vegetation is generally referred to as Valley Bushveld and forms part of the region’s dry grass-bush community. The woody layer is dominated by *A. karroo* trees which range in size from seedlings to mature trees up to 3 m in height. There are also a variety of evergreen bush species present at low density together with many succulents which, together with the low availability of fuel, has largely excluded fire.

Bush/tree clumps dominated about 65% of the study site, forming dense thickets in some areas. The understory of most of these clumps was dominated by *B. delagoense*, with few to no plants growing in the open areas between clumps. According to Jarvel and O'Connor (1999) the irradiance regime associated with bushclumps and peripheral zones (edge of the bushclumps) was 11% and 25%, respectively, of the irradiance regime in the unshaded areas between the trees/shrubs.

Soil types

Soils from under five *A. karroo* trees (1 m from canopy edge) and from five sites between trees (2 m from canopy edge) were collected from the Addo site. The particle-size distribution (percentage), pH and elemental composition (total N, Na, K, Ca, Mg) in milliequivalents (me) per 100 g of soil were measured. Nitrogen levels were determined within 48-hours of soil collection. Soil samples were analyzed using the Agricultural Research Council – Institute for Soil, Climate and Water (ARC-ISCW) laboratories in Pretoria according to methods described by the Non-affiliated Soil Analysis Working Committee (1991). Once the soils had been analysed the samples from each of the five sites under trees were combined, as were those collected from sites between the trees. The soils from under and between trees were then randomly assigned to 10 plug positions in each of three seedling trays. Thirty plantlets, which grow on the apex of the leaves and establish by dropping to the ground, were collected from mature nursery plants and mixed together. These were then placed singly on the soil in each of the 30 plugs and placed in a glasshouse under natural light at temperatures ranging between 20 and 30°C and relative humidity between 40 and 60%. Each plantlet was provided with 10 ml of tap water every 2-3 days. The trial commenced in February 2007 and was terminated in August 2007 (7 months), whereupon the length (above- and below-ground), wet weight (above- and below-ground) and number of leaves of each plant on each soil type was recorded to ascertain if there were differences in growth rates between the two treatments. A two-sample t-test was used to determine if there were any significant differences between the various growth parameters of plantlets grown in shaded versus unshaded soils (Anonymous 2003). The same analysis was used to test for differences between the shoot:root ratios of plants grown in the two soil types.

Shading

The effect of shading on *B. delagoense* plants was assessed by growing plants under different shade conditions in a glasshouse. Plantlets were collected randomly from mature nursery plants and one plantlet was placed in each of 25, 2 l pots containing a standard potting medium. Five pots were placed under each of four different shade cloth densities (20, 40, 50 and 80%) while five potted plantlets were placed in the open. The position of the pots under each shade cloth density was rearranged randomly every 10 days. The experiment was conducted in a glasshouse and initiated in December 2006 and terminated in August 2007 (9 months). Each plant was given 100 ml of water every 2-3 days. Water retention in all pots was measured at various intervals over a 48-hour period, using a tensiometer (Van Walt SMS2500S) to determine if soil moisture retention and not shading contributed to any difference in growth rates. The first measurements were taken at 13:00 (half-an-hour after watering), then 15:00 (two-hours after watering), followed by measurements taken at 12:30 the following day (24-hours after watering) and then finally 48-hours later at 12:30. At the termination of the experiment, plants were measured and analysed as above. Differences between the shoot:root ratios of the plantlets growing under the various shade cloth densities were also analysed as above.

Results

Soil types

In terms of particle size distribution, soil from under *A. karroo* trees and the unshaded areas between trees was dominated by fine and very fine sand and silt (Table 1). However, unshaded areas between the trees had a significantly higher percentage of very fine sand. The soils from under the trees had a significantly higher percentage of clay than those in the open, which would allow them to retain more moisture and nutrients than the unshaded areas between the trees. There were no significant differences (df = 9; t = -1.333; p = 0.107; paired t-test) in the mean (± SE) percentage of nitrogen in the soils under (0.082 ± 0.009) and in the unshaded (0.100 ± 0.012) areas between the trees.

The soils under the trees were significantly more acidic than those from the unshaded areas between trees and had significantly more carbon, sodium, potassium, calcium and magnesium (Table 2), which was reflected in the size of plantlets grown in these soils which were significantly longer and heavier and also had significantly more leaves (Table 3). The shoot:root ratios of plants grown in soils from the the unshaded and shaded areas were (mean ± SE) 1.01 ± 0.08 and 1.63 ± 0.12 respectively, and were significantly different (df = 24; t = -4.35; $p<0.001$; paired t-test). However, there was no significant difference in the below-ground lengths of plantlets grown in the two soil types (Table 3).

Table 1. Mean (± SE) particle size distribution (%) of soils collected under (shaded) and between (unshaded) *Acacia karroo* trees near Addo, Eastern Cape Province, South Africa

	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Fine silt	Clay
Unshaded	1.8±0.11 a	8.3±0.81 a	25.5±1.06 a	20.2±1.08 a	24.9±0.92 a	7.1±0.68 a	10.4±0.40 a
Shaded	3.1±0.32 b	9.2±0.74 a	22.5±1.16 a	15.9±0.70 b	22.3±1.24 a	8.3±1.16 a	17.8±1.63 b
F-probability	0.010	0.431	0.107	0.014	0.146	0.398	0.005

Values with the same letters within a column are not significantly different ($p < 0.01$; Fisher's protected t-test LSD)

Shading

Plantlets grown under 80% shade cloth had markedly reduced roots, and as a consequence were generally smaller and lighter than plants grown under higher light conditions (Table 4). Plantlets grown under moderate shade had significantly longer stems than those grown in direct sunlight. The below ground wet weights of plants grown under 50% shade cloth appeared to be lower than those grown under higher light intensities but the differences were not significant. The mean shoot:root ratios of the plantlets grown under 80% shade cloth in the glasshouse were significantly greater than any of the plantlets grown in direct sunlight or under 20, 40 or 50% shade cloth (Figure 1). Soil water retention was similar between all soils under the various shade cloth intensities at all times, with the exception of 30 minutes after watering, when soils under the 80% shade cloth were significantly wetter (Table 5).

DISCUSSION

Our results confirmed studies by Jarvel and O'Connor (1999) that soils under bushclumps in the Eastern Cape Province have higher levels of organic carbon and other nutrients than the surrounding grasslands. *Bryophyllum delagoense* plants grew significantly faster in soils collected from under *A. karroo* trees than in the soils collected from the unshaded areas between trees, an indication that *B. delagoense* growth rates are enhanced in nutrient-rich soils. The soils from under

Table 2. Percentage carbon, pH and levels of various elements in milliequivalents/100 grams in the soils collected under (shaded) and between (unshaded) *Acacia karroo* trees near Addo, Eastern Cape Province, South Africa.

	% Carbon	pH	Na	K	Ca	Mg
Unshaded	0.58±0.03 a	6.72±0.08 a	0.09±0.00 a	0.38±0.03 a	2.74±0.09 a	1.40±0.14 a
Shaded	1.87±0.23 b	6.07±0.14 b	0.12±0.02 a	0.76±0.11 b	7.34±0.84 b	2.65±0.23 b
F-probability	0.001	0.007	0.212	0.016	0.002	0.004

Values with the same letters within a column are not significantly different ($p < 0.01$; Fisher's protected *t*-test LSD)

Table 3. Growth parameters of *Bryophyllum delagoense* plantlets grown in soils collected from under (shaded) and between (unshaded) *Acacia karroo* trees near Addo, Eastern Cape Province, South Africa

Site soils	N	Above ground length (mm)	Below ground length (mm)	Above ground wet weight (g)	Below ground wet weight (g)	Number of leaves
Unshaded	12	50.7±2.89 a	53.8±5.66 a	1.47±0.17 a	0.089±0.01 a	15.92±0.81 a
Shaded	14	93.6±5.94 b	59.1±3.99 a	5.43±0.63 b	0.277±0.04 b	29.42±1.59 b
Probability		< 0.001	0.084	< 0.001	< 0.001	< 0.001

[1] Values with the same letters within a column are not significantly different ($p < 0.01$; Student's *t*-test)

Table 4. Effect of light intensity (various shade cloth densities and sunlight) on plant growth parameters

Shading	N	Above ground lengths (mm)	Below ground lengths (mm)	Above ground wet weight (g)	Below ground wet weight (g)	Number of leaves
Sunlight	5	147.00±13.50 a	232.20±14.14 a	28.44±6.23 a	1.72±0.46 a	46.60±4.31 a
20%	5	205.40±11.44 b	257.80±32.81 a	34.01±2.07 a	1.89±0.18 a	51.20±2.42 a
40%	5	241.00±9.64 b	232.80±13.75 a	27.86±1.45 a	1.97±0.31 a	55.20±4.07 a
50%	5	218.00±9.38 b	234.20±23.20 a	27.56±2.41 a	1.19±0.22 ab	49.00±2.88 a
80%	5	221.20±10.22 b	84.20±20.16 b	5.12±0.59 b	0.07±0.00 b	25.00±1.61 b
F-probability		<0.001	<0.001	<0.001	<0.001	<0.001

[1] Values with the same letters within a column are not significantly different ($p < 0.01$; Fisher's protected *t*-test LSD)

the trees also had significantly higher levels of clay compared to the soils from the unshaded areas. Clay and organic matter, and to a lesser extent silt contribute to the cation exchange capacity (CEC) of soils, facilitating the uptake of nutrients (Seybold et al. 2005). However, other studies have clearly indicated that *B. delagoense* can also establish and grow on soils which have significantly low levels of organic carbon, macro-nutrients, clay and silt (Witt, Nongogo and Byrne submitted). In Australia, *B. delagoense* has been recorded on a range of soils from sand to heavy clay (Batianoff and Franks 1998), on dry skeletal soils or rock faces, shallow stony soils in open forest or loose sand near the seashore (Hannan-Jones and Playford 2002). In Madagascar, *B. delagoense* has been found growing on granites and migmatites, and also on unconsolidated sands (Witt and Rajaonarison 2004). This ability to grow on a range of soils differing in particle-size distribution, pH and elemental composition is probably enhanced by the fact that *B. delagoense* exhibits a certain amount of phenotypic plasticity in that it can shift resources to root production in nutrient- or water-limited environments (Witt, Nongogo and Byrne submitted).

Table 5. Moisture (%) retention readings of the soil in pots, under various shade cloth densities, taken at various time intervals (hours) after watering, as recorded by a tensiometer.

Shading		Time intervals (hours)			
	n	0.5	2	24	48
Sunlight	5	31.52±0.50 a	31.02±1.69 a	28.68±1.08 a	17.70±0.77 a
20%	5	33.42±0.54 a	31.46±1.16 a	30.46±0.88 a	25.50±2.00 a
40%	5	34.18±0.98 ab	31.48±1.47 a	28.18±2.44 a	19.08±3.27 a
50%	5	33.56±1.16 a	31.94±1.40 a	31.12±1.47 a	23.26±1.44 a
80%	5	36.90±1.63 b	34.62±1.00 a	32.88±1.54 a	25.16±2.19 a
F- probability		0.028	0.376	0.252	0.050

[1] Values with the same letters within a column are not significantly different ($p < 0.01$; Fisher's protected *t*-test LSD)

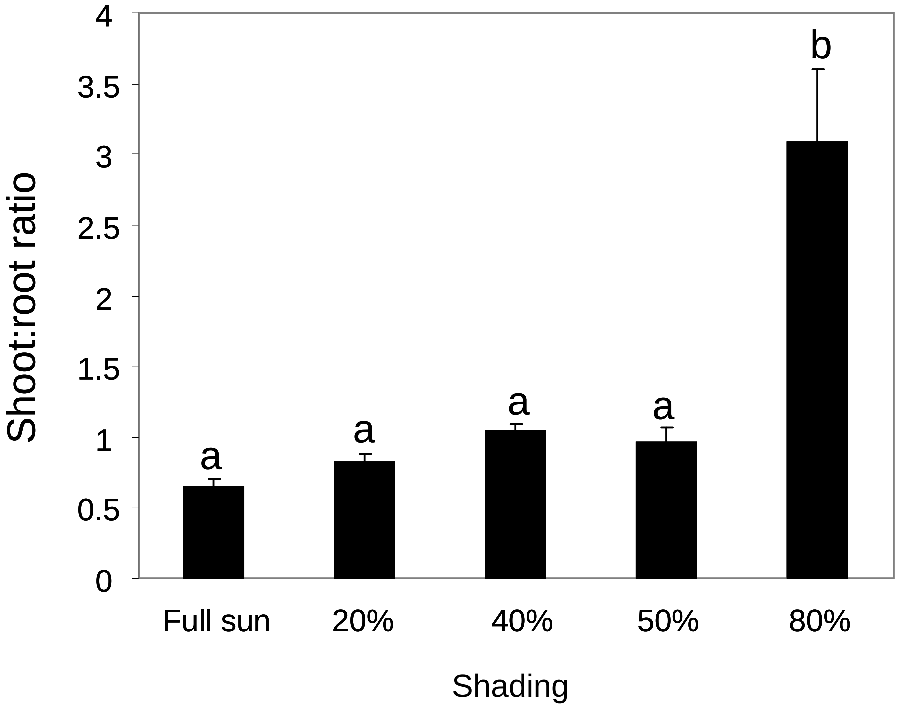
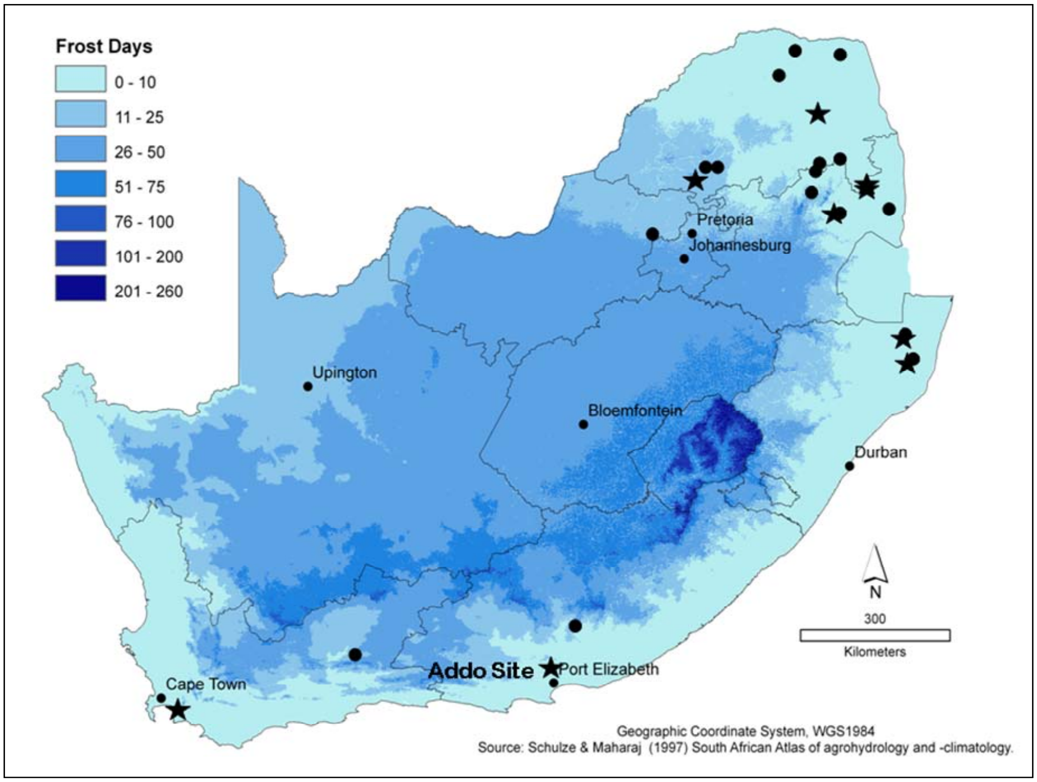


Figure 1. Mean (± SE) shoot-root ratio of *Bryophyllum delagoense* plantlets grown under different shade cloth densities and in full sun in a glasshouse. Bars with the same letters are not significantly different ($p < 0.01$; Fisher's protected *t*-test LSD)

In all growth parameters measured, plantlets grown in soils collected from under the trees performed significantly better than those grown in soils collected from the unshaded areas between trees, except for below-ground lengths where there was no significant difference. Plantlets grown in the comparatively nutrient-poor soils collected from the unshaded areas reallocated resources from leaf and stem production to root growth. This reallocation indicates growth optimization in a nutrient-limited environment (Bloom et al. 1985; Tilman 1988). Other studies (Witt, Nongogo and Byrne submitted) suggested that low soil nutrients should not preclude *B. delagoense* from establishing in the unshaded areas between trees. However, plants do not always grow under trees because of increased soil nutrients, but may be taking advantage of enhanced air humidity levels, reduced extreme temperature fluctuations and/or reduced probabilities of mechanical or herbivory damage (Holmgren et al. 1997). The invasive succulent, *Orbea variegata* (L) Haw. (Asclepiadaceae), which is also more abundant underneath shrubs than in open areas in Australia, is confined to canopies because of reduced irradiance and temperature levels and not because of increased soil nutrients under shrubs (Lenz and Facelli 2003).

Plants growing under bushclumps in the Eastern Cape Province, South Africa experience significantly lower maximum temperatures and significantly higher minimum temperatures than in the surrounding grasslands (Jarvel and O’Connor 1999). A reduction in extreme soil temperatures under canopies is crucial for succulents, because they cannot prevent over-heating through transpiration (Holmgren et al. 1997). However, this is unlikely to be the case at Addo where the highest mean monthly temperature is only 29.2°C compared to 33.3°C at Tsiombe (Sutherst and Maywald 1985), which is regarded to be the centre of the distribution of *B. delagoense* in Madagascar (Witt and Rajaonarison 2004). However, Addo gets considerably colder than Tsiombe with a mean minimum monthly temperature of 5.2°C in July compared to 11.7°C for Tsiombe (Sutherst and Maywald 1985). Higher minimum temperatures under bushclumps may therefore allow *B. delagoense* to establish in areas that experience low temperatures. More importantly, southern Madagascar does not experience any frost in contrast to the situation in South Africa where frost occurs at some of the sites where *B. delagoense* has been recorded (Schultze et al. 1997) (Figure 2), although frost frequencies at Addo are low; “frost occurs at times in winter” (South African National Parks 2001-2007). Frost occurrence may therefore be limiting a frost sensitive species such as *B. delagoense* to the “protected” areas under *A. karroo* trees at the Addo site. In southeastern Queensland and northern New South Wales, Australia, where frost occurs on average every 10-75 days per annum (Commonwealth of Australia 2005-2008) (Figure 3), *B. delagoense* mainly grows in the shaded areas under trees (Hannan-Jones and Playford 2002). However, shade is known to also improve water use due to lower sub-canopy temperatures (Durr and Rangel 2003) which could limit *B. delagoense* to the sub-canopy. Higher clay and silt levels in the soil under the canopy will contribute to greater water retention capacity compared to soils in the unshaded areas (Seybold et al. 2005). Previous studies have indicated that the competitive ability of *B. delagoense* is enhanced under high soil moisture levels (see Witt, Nongogo and Byrne submitted) and reduced moisture levels in the soil between the trees may reduce the competitive ability of *B. delagoense*. In addition, the herbaceous cover and phytomass of the grassland zone (unshaded) is significantly higher than that of the bushclump zone (shaded) in the Eastern Cape Province (Jarvel and O’Connor 1995), indicating that competition from native species may be restricting *B. delagoense* to the tree canopy. Unfortunately, the herbaceous composition at this particular site was not recorded so the effect of competition on *B. delagoense* in the field could not be confirmed. Although there are many benefits to growing under tree canopies, it is likely that *B. delagoense* is confined to these habitats due to the occurrence of frost. However, to be able to establish and grow under trees *B. delagoense* needs to be shade tolerant.

These results indicate that *B. delagoense* is phenotypically plastic to grow under the sub-canopy of trees. Most savanna trees only intercept approximately 50% of incident sunlight (Belsky 1994; Amundson et al. 1995) which supports our findings that *B. delagoense* can grow under intermediate shade but not deep shade. *Bryophyllum delagoense* allocated significantly more resources to stem production under all shade treatments with no significant concomitant increase in the number of leaves. Stem elongation is also associated with a simultaneous reduction in carbon allocation for root growth (Begna et al. 2002) but was not evident under 20 and 40% shade cloth, although there was a non-significant reduction under 50% shade cloth. Tall plants with a poorly developed root system are more susceptible to drought, but a poorly developed root system may not be critical in certain habitats, such as under trees with a dense canopy, if the soil has a higher moisture content. However, it should be noted that although many plants can tolerate low light conditions, only a fraction of them can reproduce under these conditions (Valladares and Niinemets 2008).



Source: Schulze & Maharaj (1997) South African Atlas of agrohydrology and -climatology.

Figure 2. South African frost frequency map (modified from Schultze et al. 1997) with some of the sites where *B. delagoense* was recorded in urban sites (larger black circles) and field sites (black stars) (from Witt, McConnachie and Stals submitted)

Trials were terminated before this could be ascertained but it may not be critical for a plant such as *B. delegoense* which can reproduce vegetatively by producing bulbils at the end of its leaves, which establish by dropping to the ground. In other trials *B. delagoense* allocated more resources to root growth in low-nutrient environments (see Witt, Nongogo and Byrne submitted) further supporting our contention that *B. delegoense* is plastic in that it has the ability to optimize its capacity to acquire the most limiting resource and maximize growth in a wider range of habitats.

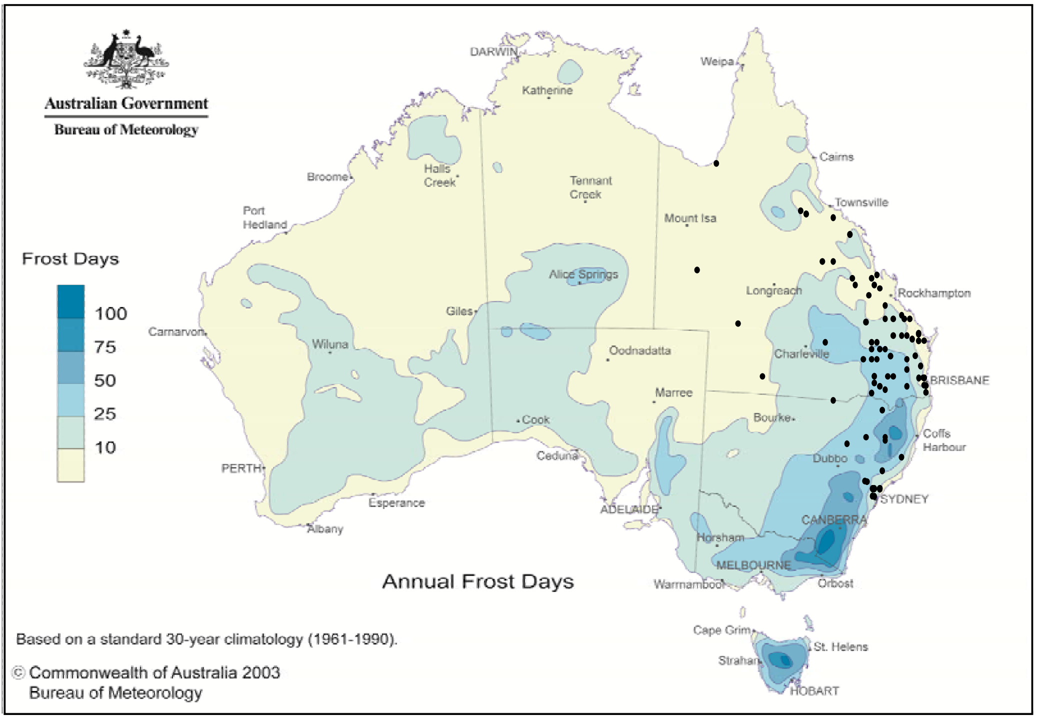


Figure 3. Australian frost frequency map with some of the sites where *B. delagoense* has been recorded (black circles) (from Australia's Virtual Herbarium 2008).

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CHAPTER 11

THE IMPACT OF FIRE, AND ITS POTENTIAL ROLE IN LIMITING THE DISTRIBUTION OF *BRYOPHYLLUM DELAGOENSE* (CRASSULACEAE) IN SOUTHERN AFRICA

Justification

Invasive species possess particular traits which predispose them to invade (Rejmánek, 1996; Rejmánek and Richardson, 1996), and ecosystems which are invaded have particular characteristics which make them susceptible to invasion (Levine and D'Antonio, 1999; Rejmánek, 1989; Rejmánek *et al*., 2005). In the previous chapters we considered traits of *B. delagoense* such as its ability to tolerate a range of soils types and shade regimes and its competitive ability and also considered characteristics of the recipient environment including increased disturbance regimes which may make Australia more prone to invasion.

Frequent disturbance, slow recovery rate, and fragmentation of successionally advanced communities promote plant invasions (Hobbs and Huenneke, 1992; Rejmánek, 1989). Ecosystems which are more prone to major disturbance events such as floods, fires and tree-falls are more likely to be invaded (Colautti *et al*., 2006; Sher and Hyatt, 1999). Fire may facilitate the invasion of many ecosystems by reducing competition from indigenous plant species and increasing availability of soil nutrients and irradiance. In fact some invasive species can promote or facilitate disturbance events such as fires to enhance their further spread (Buckley *et al*., 2007).

It is not fire *per se* that facilitates invasions but rather the time, frequency, and intensity of fire and other factors (Huenneke, 1989). Even highly fire-adapted plant communities may be vulnerable to alien competition when natural fire regimes are altered. As fire frequency increases, exotics become more important in terms of species richness and dominance (Hamilton, 1997; Huenneke, 1989).

However, fires can prevent plants spreading and have been used to reduce invasive plant populations (D'Antonia, 2000).

Prescribed burning decreased invader abundance in 20% of cases (reviewed in D'Antonia, 2000) and has been used effectively in the management of cactus species in the United States (Cave and Patten, 1984; Humphrey and Everson, 1951; Thomas, 2006). In this chapter we present the results of field trials where we determine the impact of fire on the Madagascan endemic, *Bryophyllum delagoense* and how the reduction in fire frequencies and intensities may be contributing to its invasive potential.

The number of *B. delagoense* plants surviving low and high intensity fires was recorded. Plants surviving the fire had significantly fewer leaves and plantlets while taller plants and those planted in clumps were more likely to survive the fires. This provided evidence that *B. delagoense* is extremely fire-sensitive and is unlikely to establish in fire-prone shrublands that are exposed to frequent and high intensity fires. An absence of fires may therefore facilitate its spread as has been recorded for various cactus species (Glendening, 1952). Many factors may be responsible for a reduction in fires including herbivory which competes with fires for available grass fuels and may prevent fires in some areas (Hottman and O'Connor, 1999; Thomas, 1991). It is hypothesized that an increase in disturbance as a result of overgrazing may be facilitating the invasion of *B. delagoense* in Australia and that established populations escape fires due to a lack of grass fuels. This information has made a significant contribution to our understanding of the role of fire in preventing invasions by succulents.

Ayanda Nongogo, the technical assistant at the time of the trials helped with the setting-up and monitoring of the experiment. The paper was published journal Biological Invasions 13:125-133.

The impact of fire, and its potential role in limiting the distribution of *Bryophyllum delagoense* (Crassulaceae) in southern Africa

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Abstract

Increasing emphasis has been placed on identifying traits of introduced species which predispose them to invade, and characteristics of ecosystems which make them susceptible to invasion. Habitat disturbance such as floods, fires and tree-falls may make ecosystems more prone to invasion. However, in this study the absence of fire was considered to be a factor in facilitating the invasion potential of a Madagascan endemic, *Bryophyllum delagoense*. Fire trials in South Africa killed 89 and 45% of *B. delagoense* plants in a high and low intensity controlled fire, respectively, with tall plants and those growing in clumps more likely to escape being killed. A reduction in the incidence and intensity of fires may therefore facilitate the invasion of *B. delagoense* and contribute to its invasive potential. We speculate that *B. delagoense* is more invasive in Australia because of a reduction in the frequency and intensity of fires and that fire is, amongst other factors, largely responsible for inhibiting its invasion potential in southern Africa.

Key words: Bryophyllum delagoense • Crassulaceae • fire • *Opuntia*

Introduction

Invasive alien species are considered to be the biggest threat to biodiversity and ecosystem function after habitat destruction (Mooney and Hobbs 2000). As a result increasing emphasis has been placed on research to identify traits of introduced species which predispose them to invade, and characteristics of ecosystems which make them more amenable to invasion as this is an interesting ecological question, and could facilitate screening and management of plant imports into vulnerable habitats (Lloret et al. 2005; Lambdon and Hulme 2006; Lososová et al. 2007; Theoharides and Dukes 2007). Traits of invasive species include, amongst others, rapid growth rates, phenotypic plasticity, small seed mass, short juvenile period, short intervals between large seed crops, and in many cases seed dispersal by vertebrates (Rejmánek et al. 2005). Ecosystems which are similar to those in the introduced species' native range are also more prone to invasion, especially if the climate and soil types are similar (Rejmánek et al. 2005). *Bryophyllum delagoense* (Eckl. & Zeyh.) Schinz (Crassulaceae; mother-of-millions; finger plant) has been shown to grow in a wide variety of soil types (Witt, Nongogo and Byrne submitted) and is shade tolerant (Witt and Nongogo submitted) but, disturbance factors such as floods, tree-falls and fires may also make habitats more invasible by *B. delagoense*.

Fire may facilitate the invasion of many ecosystems, but it can also inhibit and prevent plants spreading, and as a result has been used as a management tool to reduce some invasive plant populations (D'Antonia 2000; DiTomaso 2006). Prescribed burning decreased invader abundance in 20% of cases (reviewed in D'Antonia 2000). High temperature fires can cause direct damage to plant tissue through combustion, and indirect damage to the physiological processes through radiant heating (Levitt 1972). Plant tissue that is metabolically inactive or dehydrated can withstand greater heating than tissue that is metabolically active or hydrated (Whelan 1995), making succulents potentially more susceptible. Burning during a plant's active growing season therefore often results in the highest mortality rates (Di Tomaso 2006). Succulents, such as species in the

Cactaceae and Crassulaceae, retain high moisture levels throughout most of the year and as a result are generally always sensitive to fires and can therefore be controlled, provided that fuel loads are high enough to carry a fire (Thomas 1991). In this paper we investigate the sensitivity of a Madagascan endemic, *B. delagoense*, to fire.

Bryophyllum delagoense is a biennial erect and hairless succulent herb with leaves that range from 15-130 mm long and 2-6 mm wide (Henderson 2001). Each mature leaf produces plantlets which establish by dropping to the ground. This mode of vegetative reproduction allows *B. delagoense* to form dense stands composed of many immature and mature plants growing at high densities (ABR Witt pers. observ.). *Bryophyllum delagoense* has been cultivated widely throughout the world and has become naturalized in many countries, including Australia (Hannan-Jones and Playford 2002) and South Africa. Despite its long history in southern Africa, it is still largely perceived as a garden escapee in contrast to the situation in Australia where it is particularly invasive in Queensland and northern New South Wales (Hannan-Jones and Playford 2002). The small plantlets and tiny seeds within inflorescences are assumed to be vulnerable to fire, and the plant rarely resprouts if it is damaged (ABR Witt pers. observ.), unlike many fire adapted savanna plants such as *Acacia* species (Midgley and Bond 2001), or larger succulents like some *Opuntia* species (Thomas and Goodson 1992). It is hypothesized that in Australia, a low fire frequency and intensity may contribute to the invasion success of *B. delagoense*, compared to the situation in southern Africa. Controlled burns were undertaken in South Africa to quantify the impact of fire on this invasive succulent and comparisons made with the impact of fire on *Opuntia* species, many of which are invasive on both continents.

Materials and methods

Experimental burning plots

Twelve months prior to the commencement of the trial 300 *B. delagoense* plants of various sizes, in their first year of growth, were collected from the field in and around Pretoria, South Africa; potted and maintained under shade cloth and irrigation. Six months later, 260 of these plants were selected into 13 groups of 20 plants each, carefully removed from the pots with the soil in which they were growing, and placed into holes dug in the ground in 13, 10 x 10 m quadrats in a mowed grassland plot at the Rietondale campus of the ARC-Plant Protection Research Institute in Pretoria, South Africa. Efforts were made to place a range of plants of different sizes in each group and replicate that for every group, so that all 13 groups would share the same number and range of similarly sized plants. Ten plants were positioned singly at random points in each quadrat and the remaining 10 were clumped together, no further than 15 cm from each other, also in the same quadrat (20 plants per quadrat). This was done to mimic the natural distribution of *B. delagoense* infestations in the field. The plants were irrigated at regular intervals as needed during this six month period.

Two days before the trial area was burnt the grass in each quadrat was mown to ensure that there were no differences in above-ground biomass between quadrats and the above-ground length, number of leaves and plantlets of each *B. delagoense* plant was recorded. Two different fire intensities were created by burning dry oat straw on 10 of the quadrats. A high intensity fire was simulated by burning 45 kg (4.5 tonnes/ha) of dry oat straw evenly distributed on each of five quadrats; a low to moderate intensity fire from 20 kg (2 tonnes/ha) of dry oat straw on the other five; with the remaining three quadrats acting as controls (space constraints prohibited five control quadrats). Unlike the situation in a natural grassland or savanna where grass species may attain heights of well over a metre, the flammable material, in this trial was confined to 30-50cm above the ground. The straw was evenly placed around the stems of individual plants and not clumped up and around the stems and leaves. The straw was placed on the quadrats on the morning of the 6th of January 2007, and at 10h00 on the same day the first quadrat was set alight at the southeast corner, using a gas burner. Only one to two quadrats were set alight at any one time, every one of them in the southeast corner. The sequence in which treatments were burnt was determined randomly. Although it would have been preferable to burn all quadrats at the same time this was not possible due to the fire risk. The time taken to burn each quadrat was recorded during the trial. Twenty days after the quadrats had been burnt, the number of live and dead plants and the number of leaves and plantlets on those that were still alive, was recorded. The number of plants still alive was recorded again three months later.

Determination of fire intensities

There are several broad measures of fire intensity such as heat per unit area, reaction intensity and fire-line intensity (Biswell 1989). We calculated heat per unit area which measures the total energy released by a fire per unit area and Byram's (1959) fire-line intensity which measures the rate of energy released along the fire front. The former is more a measure of the severity of the fire in terms of its impact on the soil, and on longer-term plant recovery. In this respect fire intensity was calculated as:

$$FI = H \times w$$

where FI is fire intensity as heat transferred per unit area (kJ m^{-2}), H is heat yield (kJ g^{-1}), and w the mass of fuel combusted (g m^{-2}). A heat yield (H) of 16.89 kJg^{-1} was used in these calculations since this value is assumed to be constant for all fires (Catchpole 2002; Govender et al. 2006).

Fire-line intensity was estimated using Byram's (1959) fire intensity equation:

$$I = Hwr$$

where I is fire-line intensity (kW m^{-1}), H is heat yield (kJ g^{-1}), w the mass of fuel combusted (g m^{-2}), and r the rate of spread of the head fire front (m s^{-1}). The rate of spread for the head fires was estimated as:

$$r = A/(L \times T)$$

where r is the rate of spread (m s^{-1}), A the area burnt as a head fire (m^2), L the mean length of fire front (m) and T the duration of the burn (s). This method provided a single estimate of the rate of spread on each of the 10 plots.

Statistical analysis

The experiment was designed as a completely randomized design (CRD). Analysis of variance (ANOVA) was performed to determine if there were significant differences in above-ground lengths of plants in control, low and high intensity fire quadrats, two days prior to the ignition of the straw in the quadrats. The data was acceptably normal with heterogenous treatment variances. ANOVA for unbalanced data was used to test for differences between the number of leaves and plantlets on plants prior to and after the fire in control, low and high intensity fire plots. This data was acceptably normal with homogeneous treatment variances and therefore tested at the 5% level. Treatment means were separated using Fishers' protected t-test least significant differences (LSD) at the 5% level of significance (Snedecor and Cochran 1980). Data were analysed using the statistical programme GenStat for Windows (Anonymous 2003). Statistical differences between the above-ground length of plants prior to and after the fire were determined using a t-test.

Results

There were no significant differences in the mean (± SE) above-ground lengths of *B. delagoense* plants in the control (62.59 ± 3.15 cm), low (58.70 ± 2.42 cm) and high (59.31 ± 2.33) intensity fire quadrats two days prior to the ignition of the straw in the quadrats (df = 2, 257; F = 0.391; *P* = 0.676). The first quadrat was ignited at 10h00 and the last quadrat stopped burning at 11h50. The rate of spread ($m\ s^{-1}$) in the low fire intensity quadrats ranged from 0.00567 to 0.01004 $m\ s^{-1}$ (mean ± SE = 0.00793 ± 0.00078 $m\ s^{-1}$), and in the high fire intensity quadrats from 0.00873 to 0.01344 $m\ s^{-1}$ (mean ± SE = 0.01141 ± 0.000902 $m\ s^{-1}$). The mean heat transfers per unit area were 33.78 $kJ\ m^{-2}$ and 76.005 $kJ\ m^{-2}$, for the low and high intensity fires respectively. The mean fire-line intensities for the plots with a low (200 $g\ m^{-2}$) and a high fuel load (450 $g\ m^{-2}$) were 26.81 $kW\ m^{-1}$ (range 19.16-33.92, *n* = 5) and 86.75 $kW\ m^{-1}$ (66.38-102.16, *n* = 5), respectively. The minimum and maximum daily temperatures for the 20 days after the fire were 15 and 32.6ºC, respectively. The mean (± SE) relative humidity during this period was 61.22 ± 2.083% (range = 42.4-77%) while rainfall within a 24-hour period varied between 0.1 and 17.6 mm.

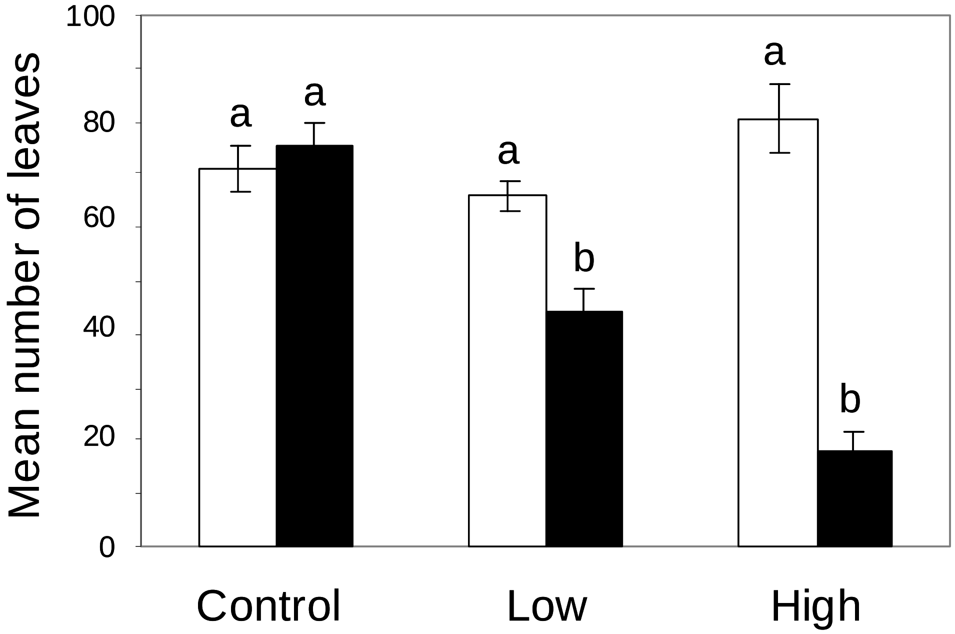

Figure 1. The mean number of leaves on surviving *Bryophyllum delagoense* plants in control (n=60), and low (n=55) and high (n=11) intensity fire quadrats before (open bars) and 20 days after (dark bars) fire, with standard error bars. Columns with the same letter are not significantly different ($P < 0.05$; Fisher's protected *t*-test LSD).

Twenty days after the fire all of the *B. delagoense* plants in the control plots were still alive while only 55 and 11% of all of the plants in the low and high intensity plots, respectively, survived the fire. Three months after the fire, 97% of plants in the control quadrats were still alive, with only 50 and 10% of the plants in the low and high fire intensity quadrats, respectively, still growing. There were no significant differences in the mean (± SE) number of leaves on plants in the control (n = 60 plants; 71.33 ± 4.36); low (n = 55 plants; 65.96 ± 3.10) and high (n = 11 plants; 80.55 ± 6.29) fire intensity quadrats prior to the fire, while those plants that survived the low and high intensity fires had significantly fewer leaves (Figure 1). Those plants that survived the fires across all quadrats also had significantly fewer plantlets (mean ± SE) in the low (162.61 ± 8.63 vs 110.51 ± 8.67) and high (185.09 ± 24.79 vs 58.73 ± 14.71) fire intensity quadrats, 20 days after the fire than before the fire (Figure 2).

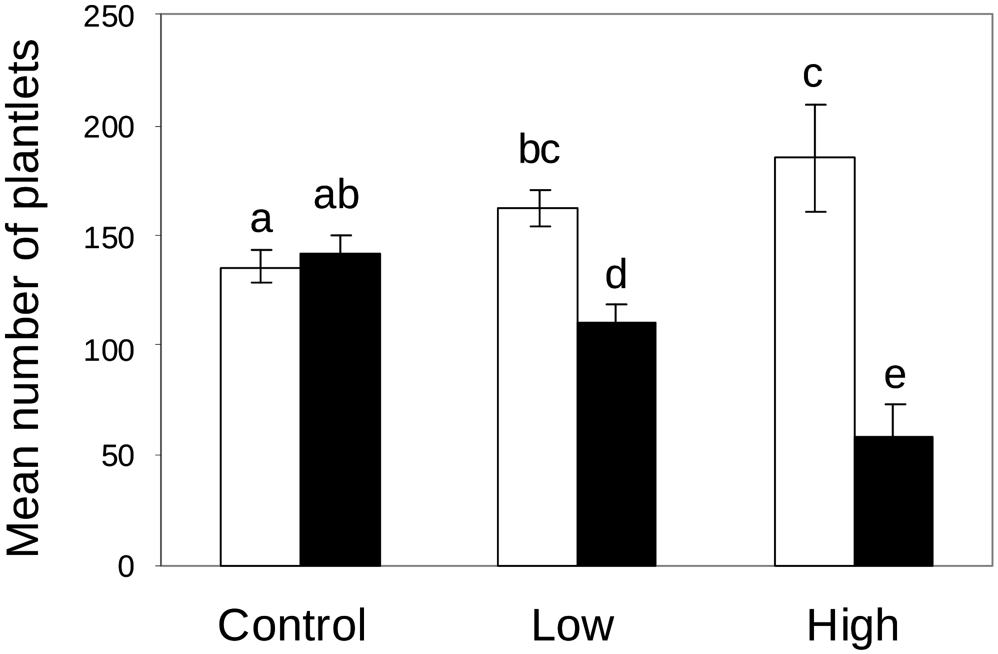

Figure 2. The mean number of plantlets on surviving *Bryophyllum delagoense* plants in control (n=60) and low (n=55) and high (n=11) intensity fire quadrats before (open bars) and 20 days after (dark bars) fire, with standard error bars. Columns with the same letter are not significantly different ($P < 0.05$; Fisher's protected *t*-test LSD).

Taller *B. delagoense* plants were more likely to survive both low and high intensity fires. Plants (mean ± SE) that survived the low intensity fires were significantly taller than those that were killed (70.058 ± 2.10 vs 47.055 ± 3.99 cm; df = 98; t = 5.353; $P < 0.05$). The same situation was observed in the high fire intensity quadrats where the 11 plants (mean ± SE) that survived the fire were significantly taller than those that were killed (73.718 ± 3.97 vs 57.53 ± 2.51cm; df = 99; t = 2.217; $P < 0.05$). The clumping of *B. delagoense* plants also seemed to have an impact on survival with 39 (71%) of the 55 plants, growing in clumps, still alive in the low intensity fire quadrats after 20 days. A similar pattern was also observed in the high intensity fire quadrats, where eight (73%) of the 11 plants that survived the fire, were growing in clumps. Plant height and the clumping of plants clearly influenced survival rates, more so in low intensity fire than high fire intensity quadrats.

Discussion

The family Crassulaceae consists of about 1 400 species of leafy succulents which predominantly occur in semi-arid to arid and rocky habitats of the temperate and subtropical zones of the northern hemisphere and southern Africa (t'Hart 1995). They are adapted to surviving in water stressed habitats because they typically exhibit Crassulacean Acid Metabolism (CAM), which is a modification of the photosynthetic carbon assimilation pathway, an adaptation to water-shortage stress (Lüttge 2004). Species which exhibit CAM, such as the Crassulaceae and Cactaceae, are not typical of savanna habitats which are dominated by C_3 and C_4 grasses and C_3 trees and shrubs (Lüttge 2004). According to Ellenberg (1981), CAM succulents struggle to establish in savanna ecosystems because they are often overgrown by grasses. CAM also does not appear to be a particularly appropriate adaptation to the seasonally variable rainfall and frequent fires of savannas (Lüttge 2004).

This is confirmed by the natural distribution of *B. delagoense*, which is restricted to the southern part of Madagascar in a vegetation type commonly known as the 'spiny desert', dominated by thorny, cactus-like plants in the family Didiereaceae as well as *Euphorbia*, *Aloe* and *Kalanchoe* species (Boiteau and Allorge-Boiteau 1995). Plants often grow in accumulated sands on granite outcrops in association with other succulents and arid-adapted species, including *Xerophyta* sp., *Senecio* spp., *Pachypodium* sp., *Euphorbia* spp., *Alluaudia* spp., *Aloe* spp., and even introduced *Opuntia* spp. (Witt and Rajaonarison 2004). Due to the dominance of succulents in the dry south there is insufficient flammable material to carry a fire and the granite outcrops act as refugia, providing additional protection against wildfires. Plants growing on rocky outcrops usually suffer very little fire damage (Steenbergh and Lowe 1977). This absence of fires in southern Madagascar is largely confirmed by MODIS fire maps (NASA/University of Maryland 2002) (Figure 3).

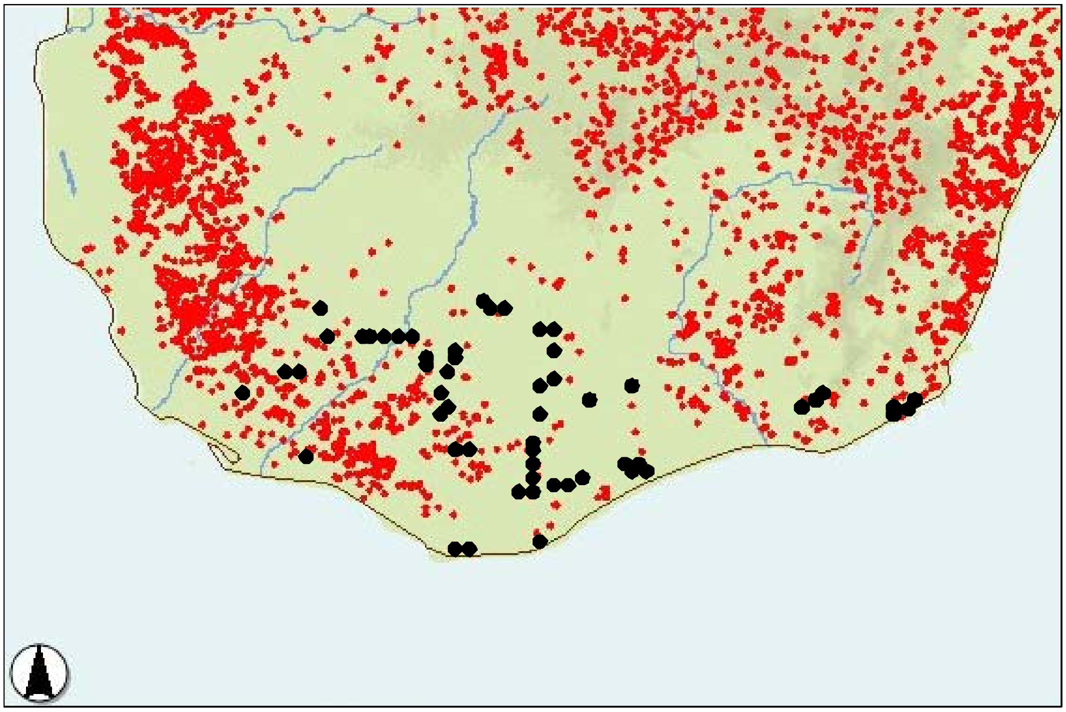

Figure3. Web fire map showing annual fire incidence in southern Madagascar in 2006 (in red) (NASA/University of Maryland 2002) with the sites where *Bryophyllum delagoense* was recorded (black circles; Witt and Rajaonarison 2004)

The sensitivity of other succulents, such as various cacti species, to fire was also confirmed by Thomas and Goodson (1992) who found that cacti only survive in refugia such as rocky outcrops, although they may be tolerant of low intensity fires. Indigenous cactus species that have increased in abundance in pastures in the United States have been effectively controlled using fire (Humphrey and Everson 1951; Cave and Patten 1984; Thomas 2006). For example, two years after a prescribed burn in southern Arizona densities of barrel cactus (*Ferocactus wislizeni* (Engelm.) Britt. & Rose), *Opuntia fulgida* Engelm., *Cylindropuntia spinosior* (Engelm.) Knuth and *O. engelmannii* Engelm. were reduced by 67, 44, 45 and 32%, respectively (Reynolds and Bohning 1956). In the Kruger National Park, South Africa, *O. stricta* is less likely to occur in areas burnt more than five times, which indicates that this species is “extremely fire sensitive” (Foxcroft et al. 2004). Indigenous succulents, including *Gasteria*, *Aloe* and *Crassula* species, are all killed by fires in South African sites (SJ Milton pers. comm.).

Despite the fact that succulents, in general, are sensitive to fires the impact on an individual plant is largely dependent on its size and also the species (Bunting et al. 1980). In this study, smaller *B. delagoense* plants were more likely to be killed by fire than taller plants, which has also been recorded for various cactus species (Dwyer and Pieper 1967; Heirman and Wright 1973), because flame height is directly related to fire intensity (Alexander 1982). Fire intensity, on the other hand, is dependent on the available fuel load which largely determines the impact of a fire. In a controlled burn in Texas a heavily grazed *Buchloe dactyloides* (Nutt.) Engelm. field had a fuel load of 1386kg/ha compared to a largely ungrazed *Hilaria mutica* (Buckl.) Beth. pasture which had a fuel load of 4836kg/ha (Bunting et al. 1980). More than 81% of *Opuntia* plants less than 30 cm in height were killed in the *H. mutica* grassland compared to only 48% in the *B. dactyloides* dominated grassland. Small succulents, like some cactus species and *B. delagoense*, are therefore more likely to survive a fire in heavily grazed pasture.

In Arizona the densities of *O. fulgida*, *C. spinosior* and *O. engelmannii* increased on grazed areas during the period from 1932-1949, from 5 to 368, 14 to 785 and 0 to 74 plants/ha, respectively (Glendening 1952). This increase was mainly ascribed to an absence of fire (Bunting et al. 1980). Herbivory therefore competes with fires for available grass fuels and may prevent fires in some areas. For example, extensive grazing in the southwestern United States, beginning in the 1820’s, coupled with improved fire suppression, progressively reduced grass cover and hence fuel loads to such an extent that fire frequency by the end of the century was reduced from about 10 to more than 50 years (Thomas 1991). As a result a number of cacti and other succulents invaded these areas. In grasslands in KwaZulu-Natal, South Africa, increases of the indigenous succulents *Aloe spectabilis* Reynolds and *Euphorbia pseudocactus* Berger and the alien *O. ficus-*

indica (L.) Mill., were ascribed to initial overgrazing and the subsequent absence of fires (Hottman and O'Connor 1999).

Once succulents like various *Opuntia* species and *B. delagoense* have established in grasslands, in the absence of fire, they become very difficult to subsequently control using fire because of their growth habit, which can possibly be seen as a fortuitous adaptation for survival in fire-prone habitats. Many *Opuntia* species form dense non-flammable clumps which largely escape fire because the outer pads of large plants shield the inner pads from intense heat (Benson and Walkington 1965). A similar situation was also observed in this trial, where *B. delagoense* plants growing at the centre of clumps were more likely to escape being killed by fire. All *Bryophyllum* species produce plantlets at the edges of their leaves, which establish by dropping to the ground. In summer, a large *B. delagoense* plant can have more than 200 plantlets attached to its leaves at any one time; consequently a single *B. delagoense* plant could be one of a large cohort of plants. More than 100 plants/m^2 have been recorded at some sites in South Africa (K. Lorentz, unpubl.). These high densities, and the fact that *B. delagoense* is allelopathic (Hannan-Jones and Playford 2002), may prevent flammable species from establishing or surviving within stands and contribute to the protection of plants in the centre of dense infestations from fire. These dense stands are relatively resistant to fire because succulent plants increase the moisture content of live fuels, potentially making it more difficult for fire to ignite and spread (Brooks et al. 2004).

In southern Africa, *B. delagoense* was only found at 11 field sites, with the largest infestation near Addo in the southern Cape where the plants were mainly growing under *Acacia karroo* Hayne (Fabaceae) trees, a micro-site dominated by other succulent species and small shrubs. In mixed bushveld, in South Africa, young *Aloe marlothii* Berger, *Euphorbia ingens* E. Mey. ex Boiss. and the introduced *Opuntia vulgaris* Mill. plants were predominantly encountered under woody canopies, where they were protected from fire (Thrash 1998). There was a significant association between being damaged by fire and growing between woody canopies for all three species (Thrash 1998). The association of *B. delagoense* with a woody canopy may therefore protect plants from fire.

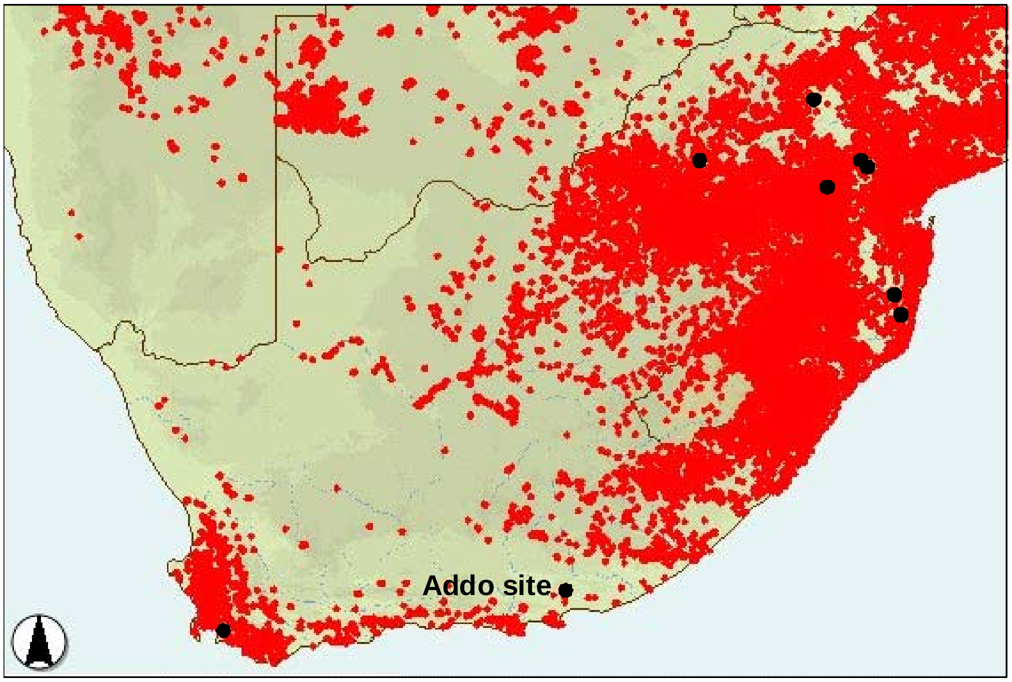

Figure 4. Web fire map showing annual fire incidence in South Africa in 2006 (in red) (NASA/University of Maryland 2002) with the sites at which *Bryophyllum delagoense* was recorded in the field (black circles; Witt, McConnachie and Stals submitted)

However, there was very little grass cover at the Addo site making it highly unlikely that the vegetation would be able to carry a fire. According to Mucina et al. (2006) the arid and semi-arid Nama and Succulent Karoo biomes of South Africa typically comprise sparse grass, widely spaced shrubs and succulents and seldom accumulate sufficient combustible fuel to support fire. This is further supported by Kerley et al. (1995) who found that there is no widespread or regular fire regime in the subtropical thickets of the southern Cape probably as a result of the low availability of fuel and high degree of succulence. This is confirmed by a MODIS fire map for 2006 which shows the low incidence of fires in the southern Cape (NASA/University of Maryland 2002) (Figure 4). This may explain why so many introduced cactus species have established in this region, including small species such as *O. humifusa*, which will probably not establish in more fire-prone habitats to the north (HG Zimmermann pers. comm.). The *B. delagoense* infestation at False Bay, on the shores of Lake St Lucia in KwaZulu-Natal, is on the ecotone between the coastal forest and the saline lake, an area unlikely to burn. The other sites were a lot closer to human habitation and would therefore, in theory, be protected from fire.

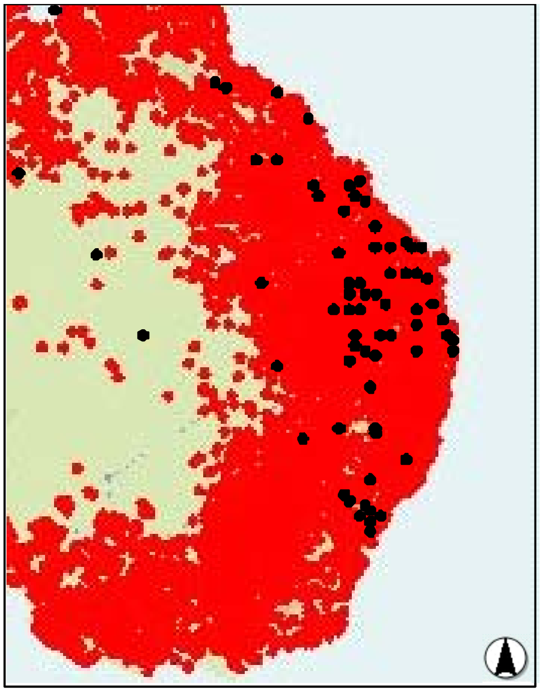

Figure 5. Web fire map showing annual fire incidence in western Australia in 2006 (NASA/University of Maryland 2002) with some of the sites at which *Bryophyllum delagoense* was recorded (black circles; Australia's Virtual Herbarium 2008)

In Australia, *B. delagoense* is common in leaf litter or other debris on shallow soils, in shady woodlands, and along roadsides and fence lines and on old rubbish dumps (Naughton and Bourke 2005). It also occurs frequently along creeks and rivers where it is spread by floodwater (Naughton and Bourke 2005). *Bryophyllum delagoense* spreads from these areas, especially after floods, and can establish in pastures that are “run down” (Naughton and Bourke 2005). Overgrazed pastures therefore facilitate the invasion of *B. delagoense*, and with 58% of the total pasturage in Queensland in a degraded or deteriorating condition (Tothill and Gillies 1992) large areas may be susceptible to invasion. In addition, introduced livestock and grazing management practices are amongst the most widespread agents of chronic modification to riparian zones in Australia (Wilson 1990; Walker 1993). High grazing pressure in areas alongside water bodies and elsewhere has led to a major reduction in perennial grass cover and a concomitant decrease in fire frequency (Wilson 2002).

It is clear that *B. delagoense* and other succulents are sensitive to fire and that the absence or reduction in fire frequency and intensity has probably contributed to their invasion potential. However, we can only speculate as to why *B. delagoense* is far more invasive in Australia than in South Africa and without detailed accounts of the incidence of fire at specific infestations this largely remains conjecture. Although satellite technology can give an indication of the occurrence of fires across a region, the resolution in the current MODIS fire maps (NASA/University of Maryland 2002) is too coarse to indicate if specific sites have been burnt. Current fire detections represent the centre of a 1 km pixel “flagged” as containing one or more active hotspots/fires within that pixel, so it is impossible to determine if a specific *B. delagoense* infestation within a 1 x 1 km area has been burnt. This is clearly demonstrated in the 2006 MODIS fire map for western Australia where it is not possible to determine if specific sites have been burnt (NASA/University of Maryland 2002) (Figure 5). This shortcoming may be resolved in future which will either provide evidence to support or reject our hypothesis that *B. delagoense* is more invasive in Australia than South Africa because of the absence of widespread high intensity fires in Queensland and New South Wales.

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CHAPTER 12

GENERAL DISCUSSION AND CONCLUSIONS

Several 19th century naturalists such as Charles Darwin, Alphonse De Candolle, Joseph Hooker and Charles Lyell, briefly mentioned invasive species in their writings which were not regarded as a major threat to biodiversity in their time (Richardson and Pyšek, 2008). It was only in 1958, with the publication of Charles S. Elton's book "The Ecology of Invasions by Animals and Plants" which for the first time focussed scientific attention on biological invasions (Richardson and Pyšek, 2008). Elton (1958) stated that biological invasions '…. are so frequent nowadays in every continent and island, and even in the oceans, that we need to understand what is causing them and try to arrive at some general viewpoint about the whole business'. Much research followed Elton's call in order to gain a better understanding of which characteristics or traits organisms possess that make them invasive, because only 1% of introduced plant species ever become invasive (Williamson, 1996). A high percentage of species in the families Amaranthaceae, Brassicaceae, Convolvulaceae, Malvaceae, Poaceae, Papaveraceae, and Polygonaceae are invasive globally, an indication that species in these families may possess certain common characteristics that predispose them to invasion (Daehler, 1998; Pyšek, 1998; Weber, 1997). The number of invasive species in the family Crassulaceae is relatively low compared to these families (Randall, 2002).

Species in the Crassulaceae occur predominantly in semi-arid to arid and rocky or mountainous habitats of the temperate and subtropical zones of the northern hemisphere and southern Africa ('t Hart, 1995). Many species in this family of leafy succulents are important in the horticultural industry and as such have been introduced as ornamentals to many countries worldwide (Eggli *et al.*, 1995). Of the approximately 35 genera and 1500 species in the Crassulaceae worldwide (Eggli *et al.*, 1995), 32 and approximately 48 species (excludes all varieties and subspecies) have been introduced to Australia (Everett and Norris, 1990) and southern Africa (Glen, 2007), respectively, an indication of their horticultural value. However, many of these introduced species have escaped cultivation with the result that globally approximately 214 species (14%) in the family Crassulaceae have been recorded as garden escapes, environmental weeds, naturalized or invasive in 35 countries or islands worldwide (Randall, 2002). According to Randall (2002) most global records for the Crassulaceae were obtained for *Bryophyllum pinnatum* (57), followed by *B. delagoense* (Eckl. & Zeyh.) Schinz (43), *Sedum acre* L. (35), *Sedum spurium* M. Bieb. (23), *Aeonium arboreum* (L.) Webb & Berthel. (22), and *Sedum album* (L.). Of the species in the family Crassulaceae introduced to Australia 81% are considered to be environmental weeds which are defined by Randall as "species that invade native ecosystems" (Randall, 2002). However, *B. delagoense* was the only species in the Crassulaceae considered to be a serious weed in Australia appearing in position 54 in an analysis of 71 invasive plant species using criteria such as invasiveness, impacts, potential for spread and socioeconomic and environmental values to rank them (Thorp and Lynch, 2000). *Bryophyllum delagoense* has also been recorded as invasive on the Galápagos Islands (Charles Darwin Foundation, 2008), French Polynesia (Lorence and Wagner, 2008), Hawaii (Wagner *et al*., 1999), Palau (Space *et al*., 2009), La Réunion (Lavergne, 2006), and New Zealand (Webb *et al*., 1988). *Bryophyllum pinnatum* has also been found to be invasive particularly on islands (Charles Darwin Foundation, 2008; Lorence and Wagner, 2008; Space and Flynn, 2001; Wagner *et al*., 1999; Webb *et al*., 1988) together with *B. daigremontianum* (Lorence *et al*., 1995). Of the 48 species in the Crassulaceae that have been introduced to southern Africa none are currently regarded as invasive although some species in the genus *Bryophyllum* are naturalized (Glen, 2002). In the United States, *B. delagoense* is naturalized in Florida, Texas and Hawaii (USDA 2007), but only its congener, *B. pinnatum* has been identified as a Category II invasive species in Florida (FLEPPC 2005).

All *Bryophyllum* species are endemic to Madagascar and differ from other genera in the Crassulaceae in that they have pendulous, bell-shaped flowers with their sepals united, and the stamens are inserted at the base of the corolla (Lucas, 2002). In addition, *Bryophyllum* species exhibit vegetative reproduction by forming adventitious buds (bulbils or plantlets) in one or more of the notches in the leaf margins; these detach, fall to the ground and grow (Lucas, 2002). A large *B. delagoense* plant can have 200 plantlets on the edges of its leaves at any one time, which drop to the ground and establish close to the mother plant or are dispersed by water and establish elsewhere. Plantlets mature after two years and die after they have flowered. Each seed capsule produces thousands of tiny seeds which are probably involved in long-distance dispersal. Characteristics associated with reproductive potential and dispersal are important correlates of invasiveness (Crawley *et al*., 1996; Richardson and Cowling, 1992; Thompson *et al*., 1995). Vegetative reproduction facilitates the establishment and short-distance dispersal of many species (Andersen, 1995; Auld *et al*., 1983; Pyšek, 1997; Thompson *et al*., 1995). Vegetative reproduction may be contributing to the invasive potential of various *Bryophyllum* species with at least six of the eight *Bryophyllum* species introduced to Australia considered to be environmental weeds (Randall, 2002). According to Herrera and Nassar (2009) *B. diagremontiana* plantlets of asexual origin showed high survival (75-100%) compared to seedlings of sexual origin (10%). In addition, asexual plantlets reproduce in less than a year compared to sexual seedlings which require a minimum of three years to reproduce (Herrera *et al*., 2011). As such seedlimg recruitment contributes little to the transient dynamics of the population (Herrera *et al*., 2011). Based on this evidence one can assume that vegetative or asexual reproduction contributes to the invasion success of *Bryophyllum* species. However, a number of introduced *Crassula* species, which don't produce plantlets or bulbils, are also considered to be naturalized and/or environmental weeds (Randall, 2002) which is an indication that vegetative production may not be the only factor in facilitating the invasion of *Bryophyllum* species in Australia. However, it is interesting to note that despite *B. delagoense*, *B. pinnatum* and *B. daigremontianum* being invasive on a number of Pacific Islands no *Crassula* or *Kalanchoe* species have been recorded as being invasive there (US Forest Service, PIER, 2009). It is unlikely that species in these taxa, which are such popular ornamentals, are not present on Pacific Islands. What is clearly required is an in depth study to resolve these anomalies. However, the fact remains that because *B. delagoense* is so easy to grow by merely planting the bulbils from the edge of the leaves, and that it produces beautiful magenta flowers in winter, when most other plant species are senescent, means that it has probably been introduced to new environments, repeatedly, over a long period of time. According to Catford *et al*. (2009) propagule pressure, which pertains specifically to the frequency of introductions, together with traits of the introduced species and characteristics of the invaded ecosystem are important correlates of invasiveness.

The date of first introduction of *B. delagoense* to Australia is unknown, but the first reported outbreak was in Chinchilla Shire, Queensland, in 1940 (Hannan-Jones and Playford, 2002). In the absence of natural enemies, it has spread over most of Queensland in the past 30 years and is particularly abundant on the Darling Downs and in central Queensland where it is spreading rapidly down river drainage systems with each flood event (Hannan-Jones and Playford, 2002). In central Queensland, it threatens the survival of the endangered bridled nail-tailed wallaby, *Onychogalea fraenata* Gould, by competing with native herbs and forbs that are the diet of this macropod (Hannan-Jones and Playford, 2002). The plant is considered allelopathic (Hannan-Jones and Playford, 2002) and consumption of its flowers by cattle has been known to cause mortality (McKenzie and Armstrong, 1986). All parts of the plant, but mainly its flowers, contain high levels of a toxin called bufadienolides which causes heart failure when consumed. Mechanical and chemical control has largely been ineffective, with the result that the then Queensland Department of Natural Resources and Mines initiated a biological control programme in 1999. This was based on the premise that the loss of natural enemies allows introduced populations to allocate increased resources to growth and reproduction, allowing them to dominate natural ecosystems. Introduced populations are often exposed to fewer species of pathogens and insect herbivores (Colautti *et al*., 2004; Mitchell *et al*., 2006) and as a result are more likely to become invasive. No species in the Crassulaceae had been targeted for biocontrol before which meant that little information was available globally on the natural enemies associated with crassulaceous species in their natural environment.

Despite the horticultural popularity of the family, a literature review (Boiteau and Allorge-Boiteau, 1995) and visits to a herbarium in Antananarivo, Madagascar, provided little information with regard to *B. delagoense* localities in southern Madagascar. There was also very little data available on the natural enemies, such as insects, mites and/or pathogens associated with *B. delagoense*, any of its congeners, or other species in the family Crassulaceae other than some insect species, mainly pests, associated with some other ornamental crassulaceous species, mainly in their introduced ranges (Dickson, 1947; Griffiths, 1976; Kroon, 1976, 1999; Marrone and Zepp, 1979; Pringle *et al.*, 1994; Schmid, 2000). As a result of this study the presence of *B. delagoense* was recorded at 75 localities in southern Madagascar, most of them previously unrecorded, all of which were surveyed at least once during four survey trips over a two-year period. Insects representing four orders, 15 families, and 23 species were collected, of which only 10 species were thought to be able to complete their development on *B. delagoense* (see chapter 3). This is similar to the number of phytophagous insect species found on small *Opuntia* species (Moran, 1980) which are morphologically quite similar to *B. delagoense*. Only those species which were considered to have the most potential as biocontrol agents were selected for further study.

Biocontrol agents are selected based on a number of traits, the most important being that they should have the ability to build-up populations rapidly in the country of introduction and ideally reach outbreak population numbers on a regular basis, be damaging and have a narrow host range. Agents with multiple generations per year will be favoured because they can build-up numbers more rapidly than univoltine insects, clearly a very beneficial attribute especially when undertaking laboratory trials. However, even multivoltine insects may fail to build-up numbers rapidly, or even establish, because of interactions with environmental factors such as climate and parasitoids, predators and competitors in the country of introduction (Wapshere, 1985; Blossey, 1995a). Climatic tolerance can possibly be predicted from the distribution of the agent in the country of origin; an agent with a wide distribution may be better adapted to a range of climatic conditions compared to an agent with an extremely narrow distribution. Based on the knowldege gained about the target species and its associated natural enemies from field surveys and other criteria three of the insects collected on *B. delagoense* in Madagascar, *Osphilia tenuipes* (Fairemaire) (Coleoptera: Curculionidae), *Rhembastus* sp. (Coleoptera: Chrysomelidae), and *Eurytoma sp.* Neser (Hymenoptera: Eurytomidae) were selected for further studies in South Africa to determine their biology and laboratory host range. Prior to this study nothing was known about the biology and host range of any of these species, with *E. bryophylli* only recently being described (Neser, 2008); *Rhembastus* sp. remains undescribed. Surveys of the insects associated with *B. delagoense* and some of its congeners in Madagascar and subsequent biology and host range studies have therefore made a significant contribution to scientific knowledge.

Despite indications that *O. tenuipes* has a narrow field host range (it was only found on *B. pubsecens* Baker in the field), no-choice and multiple choice trials in quarantine revealed that it could also oviposit and develop to adulthood on seven non-target species in the family Crassulaceae, without an obvious loss of fitness on four of those species. Despite these results, *O. tenuipes* is considered to have potential for release against *B. delagoense* in Australia because the continent has very few native Crassulaceae which could potentially serve as alternative hosts in the field. Australia has only eight indigenous species of Crassulaceae, all belonging to the genus *Crassula* with *K. crenata*, the only representative in the genus Kalanchoe in Australia, being geographically isolated from *B. delagoense* infestations (Tölken, 1981). However, there is also some doubt as to if *K. crenata* is native or not.

Favourable attributes of the weevil, *O. tenuipes*, as a biocontrol agent, include ease of culturing, multiple generations per year, and high levels of damage inflicted on *B. delagoense* under laboratory and field conditions. The fact that *O. tenuipes* was present throughout the range of *B. delagoense* in southern Madagascar indicates that it may be tolerant of a wide range of climatic conditions, another beneficial trait. Curculionids have also featured extensively in the biological control of weeds in the past (Julien and Griffiths, 1998). Some of the most successful biocontrol agents have been weevils including *Neohydronomus affinis* (Hustache) on *Pistia stratiotes* (Araceae; water lettuce), *Stenopelmus rufinasus* Gyllenhal on *Azolla filiculoides* Lamarck (Azollaceae; red-water fern), various *Melanterius* species on Australian acacias (Fabaceae; wattles), and *Neodiplogrammus quadrivittatus* (Olivier) on *Sesbania punicea* (Cav.) Benth. (Papilionaceae; red sesbania) (Olckers, 1999).

The phytophagous wasp *E. bryophylli* was found throughout the natural distribution of *B. delagoense* in southern Madagascar and was also imported into quarantine laboratories in South Africa for life history studies and preliminary host-range testing. Initially there was some confusion because another eurytomid, extremely similar in morphology to *E. bryophylli*, was also introduced into quarantine from Madagascar together with infested plant material. This eurytomid remains unidentified but studies revealed that it was probably a parasitoid of *E. bryophylli*, an interesting finding. *Eurytoma sp.* females deposit their eggs in the plantlets (bulbils), leaves and/or seedling stems and the larvae complete their development in the plant. There are five larval instars and pupation occurs in pupal chambers excavated within the plant. Adults emerge from plants 5–10 days after pupation and are short-lived. Preliminary host range trials revealed that *E. bryophylli* is oligophagous, with larvae being able to complete their development on three other species tested in the Crassulaceae, all in the genera *Kalanchoe* and *Bryophyllum*. Further support for its potential as a biocontrol agent is evident from other studies, where congeneric species have been considered or have been utilized for the biological control of invasive plants. For example, the seed-feeding *Eurytoma attiva* (Burks) has established and is a highly effective agent for the control of *Cordia curassavica* (Jacquin) Roemer & Schultes (Boraginaceae) in Mauritius, Malaysia and Sri Lanka (Julien and Griffiths 1998) while *E. cattleianii* Thuróczy & Wikler and *E. psidii* Thuróczy & Wikler are both being considered for the control of *Psidium cattleianum* Sabine (Myrtaceae) in Hawaii (Wikler and Smith, 2002). Several *Eurytoma* species were at one stage being considered for the control of *Striga* species (Orobanchaceae) (Bashir, 1987) while *Tetramesa romana* Walker has been selected as a potential biocontrol agent for *Arundo donax* L. (Poaceae) in North America (Goolsby and Moran, 2009). *Tetramesa romana* could only complete its development on the target species and *A. formosana* Hack. (Goolsby and Moran, 2009) demonstrating the restricted host range of many phyophagous species in the family Eurytomidae.

In some cases, the potential of an insect to be an effective biocontrol agent can also be inferred from the impact that congeneric species have as pests on cultivated crops. Crops often comprise monocultures, much the same as introduced invasive plants. In many cases crop pests are accidentally introduced in small numbers, but manage to establish readily and escape intensive predation and parasitism in their introduced range. The characteristics of many pest species may therefore render certain congeneric species attractive as biological control agents (Syrett *et al.*, 1996), provided that they are monophagous or oligophagous. For example, *Eurytoma amygdali* End., a major pest of almonds, is apparently strictly monophagous in Israel (Plaut, 1971), while *E. onobrychidis* Nikolskaya, a pest of sainfoin, *Onobrychis viciifolia* Scop. (Fabaceae), in many countries, including the USSR, has been reported to destroy 30–90 % of some seed crops (Richards and Hanna, 1982). Furthermore, *E. onobrychidis* is apparently monophagous and less than 1 % of the insect population is parasitized in its introduced range in Canada (Richards and Hanna, 1982). Other *Eurytoma* species that are pests of cultivated plants include *E. tumoris* Bugbee on *Pinus sylvestris* L. (Pineae) in the United States (Stark and Koehler, 1964) and *E. orchidearum*

(Westwood) on orchids in Hawaii and other countries (Best, 1952). Based on the damage inflicted on their host plants and the narrow reported host ranges of many *Eurytoma* species, it seems likely that *E. bryophylli*, collected on *B. delagoense*, has considerable potential as a biological control agent.

Another insect species considered as a potential biocontrol agent for *B. delagoense* is the flea beetle *Rhembastus* sp. Favourable attributes of the beetle include relative ease of culturing, multiple generations per year, and high levels of damage inflicted by the adults, which feed on the plantlets produced at the end of each leaf, and by the root-feeding larvae. The adults therefore have an impact on the reproductive potential of the plant, and larval feeding on the roots hampers the uptake of water and may even facilitate secondary infections by pathogens. Despite indications from field surveys in Madagascar that *Rhembastus* sp. has a narrow host range, preliminary no-choice and multiple-choice trials in quarantine revealed that it could complete its development on five non-target species in the Crassulaceae. Despite this *Rhembastus* sp. has a sufficiently narrow host range to be considered for further testing as a potential biocontrol agent in Australia. Adults and/or feeding damage was also evident at 53% of the known *B. delagoense* sites surveyed across the whole of southern Madagascar indicating that it may be tolerant of a wide range of climatic conditions, and therefore likely to establish if released in Australia. If released, it will join other highly successful root-feeding chrysomelids such as *Longitarsus flavicornis* (Stephens), *L. jacobeae* (Waterhouse), *L. aeneus* Kutschera, *L. echii* Koch and *L. albineus* (Foudras) (Julien and Griffiths, 1998), all of which have been released in Australia for the control of various invasive plant species. More recently another root-feeding chrysomelid *Longitarsus bethae* Salvini & Escalona was released in South Africa for the control of *Lantana camara* L. (Verbenaceae) (Simelane, 2005).

The host ranges of all three potential biocontrol agents collected on *B. delagoense* in Madagascar were similar with the ability to develop on other *Bryophyllum* species and also on some *Kalanchoe* species. This provided the impetus to look at the natural enemies associated with *B. delagoense* in southern Africa because this introduced species should, in theory at least, have acquired natural enemies from *Kalanchoe* species native to mainland southern Africa. Many introduced plant species never become invasive due to the presence of multiple barriers including losses caused by generalist or pre-adapted native natural enemies (Colautti *et al.*, 2004; Crawley, 1987; Cronk and Fuller, 1995; Richardson *et al.*, 2000). Introduced species are likely to acquire natural enemies from closely related plant species in the country of introduction (Colautti *et al.*, 2004; Hokkanen and Pimentel, 1989).

There are a large number of species in the Crassulaceae present in southern Africa (Eggli *et al.*, 1995), which are hosts to a multitude of natural enemies (and which were unknown prior to this study) that may be pre-adapted to feed and develop on the introduced *B. delagoense*.. The probability of the introduced *B. delagoense* acquiring new natural enemies in southern Africa is therefore greater than in Australia which only has eight indigenous species in the genus *Crassula* (Tölken 1981). Newly acquired natural enemies may therefore be reducing the fecundity and growth of *B. delagoense* in Africa and as a result limiting its spread. This may be one of the reasons why *B. delagoense* is so invasive in Australia.

The evolution of increased competitive ability (EICA) hypothesis (Blossey and Nötzold, 1995) argues that exotics long liberated from their natural enemies should lose the costly traits that confer resistance to their native natural enemies. Exotics can re-allocate resources from the maintenance of resistance to traits, such as size and fecundity. Liberation from natural enemies may result in exotics evolving in ways that enhance their performance in recipient communities. If this hypothesis is correct then *B. delagoense* is more likely to be invasive in Australia where it has only acquired two natural enemies (MA Hannan-Jones, Queensland Department of Primary Industries and Forestry, personal communication). It would be interesting to monitor the impact of *Scirtothrips aurantii* Faure (Thripidae), which was recently accidentally introduced to Australia (Manners and Dhileepan, 2005). This insect appears to be restricted to species in the Crassulaceae (Manners and Dhileepan, 2005) and is particularly damaging to *B. delagoense*, reducing bulbil production and growth rates (A.B.R. Witt, personal observation).

In chapter 7 I demonstrate that introduced species with closely related congeners are more likely to acquire pre-adapted natural enemies. This is supported by evidence from the United States and Australia where the extent of *B. delagoense* invasions is correlated with the phylogenetic relatedness to crassulaceous species in each region. Pre-adapted and/or generalist natural enemies may therefore be preventing *B. delagoense* from becoming invasive in southern Africa. One such natural enemy found in southern Africa, *Alcidodes sedi*, was selected for further study as a potential biocontrol agent for *B. delagoense* in Australia.

Alcidodes sedi larvae and/or adults were found at 20 of the 31 *B. delagoense* sites surveyed in South Africa and Botswana during this study. The weevil was also collected in cultivation or in the field on *B. proliferum*, *B. pinnatum*, *B. daigremontianum*, *K. longiflora*, *C. orbiculata,* and *Kalanchoe sexangularis*. Of the native plant species surveyed in the field *A. sedi* was only found on *K. longiflora* (it was not found in the field on any of the other native species), indicating that this species is possibly the primary host. In laboratory host range trials *A. sedi* could complete its development on all of the *Bryophyllum* species tested, with the exceptions of *B. gastonis-bonnieri* and *B. fedtschenkoi*. Development also occurred on *Kalanchoe sexangularis*, *K. rotundifolia* (Haw.) Haw.*,* *K. blossfeldiana* (von Poellnitz) Boiteau, *C. orbiculata,* and *Echeveria* sp. hybrid. There was no development on *K. beharensis*, any of the *Crassula* species, *Portulacaria afra* (Portulacaceae), or *Senecio barbertonicus* (Asteraceae). This study has demonstrated that phytophagous insects which feed and develop on introduced plant species can have a restricted host range, and be damaging, and as a result can be considered as biological control agents.

According to Hokkanen and Pimentel (1989), the probability of biocontrol success in using an insect agent that originates from a congeneric host and that is not naturally associated with the target would be approximately 75% greater than with the so-called ‘old-association’ approach, in which the agent and target plant have a long co-evolved association. Based on this finding and considering that *A. sedi* is also damaging one could assume that *A. sedi* could potentially be more successful as a biological control agent for *B. delagoense* than *O. tenuipes*, *E. bryophylli* and *Rhembastus* sp. This is based on the lack of co-evolved homeostasis, in which the plant is poorly adapted to cope with herbivory from the new insect associate.

The hypothesis of co-evolved homeostasis was rejected by Goeden and Kok (1986) and although Ehler (1992) acknowledged that new associations between phytophagous insects and host plants may have some potential in biological control he was uncomfortable with the explanation of co-evolved homeostasis and suggested that alternative hypotheses be considered for the success of new associations. The co-evolved homeostasis theory also assumes that the niche or plant part attacked by the ‘new associate’ is not attacked by a phytophagous insect and/or mite in its native range. *Osphilia tenuipes* and *A. sedi* are both stem-boring weevils which means that *B. delagoense* has probably evolved defenses to deal with weevil stem-boring, so the ‘new associate’, *A. sedi*, would not necessarily be more effective.

However, exotic plants long liberated from their natural enemies, such as *B. delagoense* in Australia, should be less resistant to attack, based on the increased competitive ability hypothesis (Blossey and Nötzold, 1995), which means that *A. sedi* should theoretically be as effective as *O. tenuipes*, irrespective of the former being a “new association” provided that both agents are similar in all other respects, which is highly unlikely. Predicting potential success is problematic and depending on how much information is available on both species it remains difficult to predict what impact, if any, parasitoids, predators, and/or competition may have on both agents. On the hand one could ‘hedge ones bets” by releasing both weevils which could increase the potential for control if both agents complemented one another or it could result in the competitive exclusion of one of the two species which may be problematic especially if the most effective agent is the one that is displaced.

Denno *et al.* (1995) found that there were competitive interactions amongst biocontrol agents in 91% of the 45 examined experimental studies between two species. This could be as a result of direct competition for the same resource or indirect suppression of a resource by one agent required by another (foliage-feeder reducing flowering) (Denno *et al*., 1995). According to Denno *et al*., (1995) phytophagous insects were more likely to compete if they were closely related, introduced, aggregative, and fed on discrete resources. The competitive exclusion principle predicts displacement of one of two ecological homologues from the same habitat (Hardin, 1960). However, two agents released for the control of *Lythrum salicaria* (Lythraceae; purple loosestrife) in the United States, *Galerucella calmariensis* L. and *G. pusilla* Duftschmid (Chrysomelidae), share their distribution on a continental, regional, and local scale including the same ecological niche on their host plant (Blossey, 1995b). This co-existence has been ascribed to both agents having identical competitive abilities (Blossey, 1995b). In other cases parasitoids have been known to facilitate the coexistence of strongly competing host species (Commins and Hassell, 1976, 1987) while in others host species tend to be reduced in abundance and even excluded from the community by other host species because of a shared natural enemy (Lawton, 1986). Predicting the outcome of competitive interactions in the country of introduction can therefore be problematic.

An attempt was made to ascertain, in the absence of natural enemies, if *A. sedi* and *O. tenuipes* could co-exist on the same *B. delagoense* plant but unfortunately the results were inconclusive. Although both agents may not be able to develop on the same plant they could possibly co-exist in the same area because the larger

A. sedi can only develop in large-stemmed *B. delagoense* plants whereas *O. tenuipes* can develop in large and small plants. In this situation co-existence would only be possible if *A. sedi* was a superior competitor. All insect species develop within certain temperature envelopes (Robertson *et al*., 2008) and both of these agents will be no different. Competitive intereactions between *A. sedi* and *O. tenuipes* may be avoided if they have disparate thermal physiologies (degree day requirements) – they may establish in different regions in Australia based on their thermal tolerances. It is recommended that studies be undertaken to determine the thermal physiology of both species prior to introduction. Having two agents occupying the same niche also provides researchers with a possible alternative should *O. tenuipes* be released first and fail to establish. There are also other good reasons for considering a number of agents for the control of *B. delagoense*. The highest levels of biocontrol 'success' have been achieved using multiple agents (Baars and Heystek, 2003; DeBach, 1964; Dennoth *et al*., 2002; Hoffmann and Moran, 1998). This is as a result of agents "working together" and providing a cumulative or synergistic effect (Harris, 1981; Hoffman and Moran, 1998) or because there is a greater probability that at least one of the agents that is released will be effective (Denoth *et al*., 2002). However, Myers (1985) is of the opinion that only one or two agents have been responsible for most of the damage to target species when multiple agents have been released. McEvoy and Coombs (1999) also caution against the environmental risk of releasing many agents.

The absence of a similar damaging insect such as *A. sedi* and others may be contributing to the invasiveness of *B. delagoense* in Australia. However, there are a host of other biotic factors which may be facilitating the invasion of Australia by *B. delagoense*, one of which is the role of competition. Competition from resident species is a chief mechanism for biotic resistance to invasion (Catford *et al*., 2009). However, disturbance events may reduce this competition from native plants, thus increasing the opportunity for invasive species to establish and spread (Catford *et al*., 2008). Abiotic characteristics of the invaded ecosystem may also contribute to invasion (Catford *et al*., 2009), including both climatic and edaphic conditions, which have long been regarded as a fundamental determinant of the potential distribution of introduced plant populations (Mitchell *et al*., 2006). Introduced species that do not have specific soil or mineral requirements would have the ability to establish and spread on a wide range of soil types within their introduced ranges (Huenneke *et al*., 1990) provided that the mycorrhizal symbionts required for growth are present (Pringle *et al*., 2009). The fact that *B. delagoense* is invasive in Australia and not South Africa, despite a much longer residence time in southern Africa, may indicate that factors other than plant species traits, but characteristics of the recipient environment, or a combination of both, may best explain its invasiveness in Australia.

Laboratory trials clearly indicated that *B. delagoense* has the ability to grow on a range of soil types. This ability to tolerate sand and clay soils, varying in nutrient levels, is a beneficial species trait (Huenneke *et al*., 1990) which will enable *B. delagoense* to establish and spread in a wide variety of habitats throughout the world, provided that other factors essential for its growth and reproduction are met, including reduced competition from native species in its country of introduction (Catford *et al*., 2009). *Bryophyllum delagoense* growth was clearly influenced by competition from a grass species but less so in clay soils when water was not limiting. Where competition from other species is low, this study suggests that the invasion success of *B. delagoense* can be attributed to its ability to tolerate a wide range of soil types. This is in agreement with other studies which have found that disturbance can increase resource availability, which has long been associated with invasion (Elton, 1958; Rejmánek and Richardson, 1996) provided that the introduced plant has sufficient phenotypic plasticity to adapt to the available edaphic conditions (Mitchell *et al*., 2006). Reduced competition from native species as a result of disturbance events such as overgrazing, combined with phenotypic plasticity may be facilitating the invasion success of *B. delagoense* in Australia.

Although *B. delagoense* is not a particularly strong competitor it has the ability to colonize disturbed areas including frost-prone areas in Australia and South Africa. Intuitively a frost-sensitive species, such as *B. delagoense*, could never become invasive in a frost prone area. However, it is assumed that *B. delagoense* has been able to establish in frost-prone areas by growing in the shade under frost-hardy trees. The ability to grow under different light intensities could therefore be considered as an important attribute of an invasive plant species, enabling establishment in a wider range of habitats (Lososová *et al*., 2007; Parendes and Jones, 2000). Shade tolerance is especially important for short weed species (Lososová *et al*., 2007), such as *B. delagoense*, which are often overtopped by larger plants. This ability to grow in direct sunlight or shaded situations is common in plants which exhibit phenotypic plasticity. *Bryophyllum delagoense* appears to optimize growth under moderate shade by shifting resource allocation to stem production, allowing it to maintain constant fitness, which is inferred but not demonstrated in this study, in frost-prone environments by growing under tree canopies. In the field, higher nutrient levels in the shade under trees may also compensate for lower light levels. This ability to tolerate moderate shade will allow *B. delagoense* to invade frost-prone areas provided that there are trees and shrubs for protection. This is a beneficial attribute and supports the contention that phenotypic plasticity contributes to the success of many invaders (Rejmánek *et al*., 2005). It also highlights the difficulties in mapping the potential distribution of invasive plant species, especially if their biology has not been well studied and documented. The potential distribution of *B. delagoense* is probably far greater than initially predicted based on the fact that it can maintain constant fitness in frost-prone areas by growing in shaded environments under trees and in nutrient-limited environments. Plants which exhibit phenotypic plasticity are therefore more likely to become invasive yet this aspect is never explored when considering the importation of a particular plant species.

The contribution of disturbance, such as overgrazing, to the invasion success of *B. delagoense* in Australia has been mentioned. However, invaded ecosystems have a host of other characteristics which make them susceptible to invasion (Levine and D'Antonio, 1999; Rejmánek, 1989; Rejmánek *et al*., 2005). The frequency of major disturbance events, slow recovery rate, and fragmentation of successionally advanced communities promote plant invasions (Rejmánek, 1989; Hobbs and Huenneke, 1992). Ecosystems which are more prone to major disturbance events such as floods, fires and tree-falls are more likely to be invaded (Sher and Hyatt, 1999, Colautti *et al*., 2006). Fire may facilitate the invasion of many ecosystems by reducing competition from indigenous plant species and increasing availability of nutrients and irradiance. It is not fire *per se* that facilitates invasions but rather the time, frequency, and intensity of fire, and other factors (Huenneke, 1989). Even highly fire-adapted plant communities may be vulnerable to alien competition when natural fire regimes are altered. In some environments, as fire frequency increases, exotics become more abundant in the landscape in terms of species richness and dominance (Hamilton, 1997; Huenneke, 1989). However, fires can also inhibit and even prevent plants spreading and in some cases have been used as a management tool to reduce invasive plant populations (D'Antonia, 2000). Prescribed burning decreased invader abundance in 20% of cases (reviewed in D'Antonia 2000) and has been used effectively in the management of cactus species in the United States (Humphrey and Everson, 1951; Cave and Patten, 1984; Thomas, 2006).

The absence of fire may facilitate the spread of *B. delagoense*. *Bryophyllum delagoense* plants grown in field plots at high and low densities were exposed to low and high intensity fires and survival recorded after 20 days when it was found that all of the *B. delagoense* plants in the control plots were still alive while only 55 and 11% of the plants in the low and high fire intensity plots, respectively, survived the fire. Plants that survived the fire had significantly fewer leaves and plantlets while taller plants and those planted in clumps were more likely to survive the fires. This provided evidence that *B. delagoense* is extremely fire-sensitive and is unlikely to establish in fire-prone shrublands that are exposed to frequent and high intensity fires. An absence of fires may therefore facilitate its spread, as has been recorded for various cactus species (Glendening, 1952). Many factors may be responsible for a reduction in fires including herbivory which competes with fires for available grass fuels and may prevent fires in some areas (Thomas, 1991; Hottman and O'Connor, 1999). It is hypothesized that an increase in disturbance as a result of overgrazing may be facilitating the invasion of *B. delagoense* in Australia and that established populations escape fires due to a lack of grass fuels. However, further experimentation is needed to show the relationship between grazing intensity and fire on *B. delagoense* population dynamics.

The study demonstrates that disturbance may be one of the main factors contributing to the invasion success of *B. delagoense* although only the role of fire was studied in any detail. Trials have indicated that *B. delagoense* is a weak competitor which means that invasions can be inhibited by ensuring that grass cover is maintained. In this regard it has been suggested that introduced African grasses be planted because they are more resistant to grazing, and hence more likely to prevent or inhibit *B. delagoense* invasions. Trials have also shown that *B. delagoense* is extremely fire sensitive which means that fire can be employed as a management strategy. However, in order to be effective the fuel load will have to be relatively high, otherwise taller plants and those growing in clumps will not be killed. Unfortunately, as a result of overgrazing there is not enough fuel to carry a fire which means that pasture management remains the primary challenge in managing this weed.

This study has been an attempt to fulfill the request by Catford *et al*. (2009) that a holistic approach be required in gaining an understanding of the factors responsible for plant invasions. To date most studies have been rather piecemeal in trying to understand why particular plant species are invasive. The holistic approach suggested by Catford *et al.* (2009) examines the influence of propagule pressure, abiotic characteristics and biotic characteristics simultaneously and, in so doing, enables their relative influence to be ascertained from the outset. In this study I have attempted to do just that, by identifying as many characteristics as possible to explain why *B. delagoense* is invasive in Australia but not in southern Africa. Biological control research provides an ideal platform to undertake these studies because they are initiated in the country of introduction followed by brief survey trips to the weeds country of origin. This gives biocontrol researchers an ideal opportunity to gain valuable insights into the biology and phenology of the target species, its distribution and that of its associated natural enemies, in the native and introduced range. These findings can make a valuable contribution to the implementation of an integrated management plan which includes chemical, mechanical, cultural, and biological control.

Very few studies have attempted to test a range of theoretical considerations in trying to explain the distribution and abundance of an invasive plant on two continents. Surveys in Madagascar and subsequent host range testing revealed that some insects collected on *B. delagoense* in southern Africa are pre-adapted to feed and develop on some *Kalanchoe* species. This subsequently led to surveys in southern Africa where a host of insects were found feeding on *B. delagoense* supporting the theory that an introduced plant is more likely to acquire natural enemies from congeners and depending on their impact less likely to become invasive. However, the acquisition of novel natural enemies is not the only factor inhibiting the invasive potential of an exotic plant. A plant which is adapted to grow in a range of soils and exhibits phenotypic plasticity is more likely to maintain constant fitness throughout its distribution. Exotic plants which are poor competitors are less likely to become invasive in their introduced range unless disturbance such as overgrazing reduces the competitive ability of native species. A reduction in grass cover will also facilitate the invasion of fire-sensitive species. All of these factors and others facilitate or inhibit the invasive potential of every introduced species. This said a factorial experimental design would have contributed immensely to my study because it may have helped in unraveling the multiple and interacting causes of the invasion of *B. delagoense* and would be a logical next step.

There have been numerous attempts to predict the invasion potential of introduced species which have culminated in the development of various Weed Risk Assessment's (WRA), such as the one developed for use in Australia and New Zealand (Pheloung *et al*., 1999). The WRA asks 49 questions based on the main attributes and impacts of weeds. The questions are divided into three sections: (i) history/biogeography which includes questions related to the documented distribution, climate preferences, history of cultivation or weediness elsewhere in the world; (ii) undesirable attributes which looks at plant characteristics such as toxicity of the plant, or its growth habit; and (iii) biology/ecology which asks questions pertaining to the attributes that enable a taxon to reproduce, spread and persist (Pheloung *et al*., 1999). The predictive ability of the WRA was assessed testing introduced plants in Florida (Gordon *et al*., 2008). The WRA rejected 92% of test species that have been documented to be invasive in Florida and correctly accepted 73% of the noninvaders (Gordon *et al*., 2008), clearly a useful predictive tool. However, the predictive ability of the WRA is severley compromised if there is no information pertaining to the invasiveness of an introduced plant elsewhere, its biological traits and characteristics of the recipient environment. Assuming that *B. delagoense* was not invasive anywhere in the world and based on its long history in its introduced range in southern Africa, where it has failed to become invasive, it is highly likely that a WRA would not have rejected its importation into Australia.

This study has clearly shown that many factors contribute to the invasive potential of an introduced species of plant. Unfortunately there are insufficient resources, especially in developing countries, to undertake in-depth studies on the biology of a plant and characteristics of the environment which may facilitate invasion. Regrettably this means that many species will continue to be introduced to new regions and become invasive because of inadequate information availability. This may not necessarily apply to plant species which are currently invasive eslewhere because that information is, in most cases, readily available but it certainly will apply to those plant species that have recently been developed for the horticultural industry. Importers of all plant species, which are not currently present within a country or region, or new varieties of species which are present, should be obligated to fund all research to ascertain if a particular species or variety has the potential to become invasive or not.

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