Biological control endeavours against Australian myrtle, *Leptospermum laevigatum* (Gaertn.) F.Muell. (Myrtaceae), in South Africa

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In South Africa, two imported insect species have been used in attempts to control invasive Australian myrtle trees, *Leptospermum laevigatum* (Gaertn.) F.Muell. (Myrtaceae): a bud-galling midge, *Dasineura strobila* Dorchin (Diptera: Cecidomyiidae), which was inadvertently introduced, possibly in the mid-1980s, and a leaf-mining moth, *Aristaea (Parectopa) thalassias* (Meyrick) (Lepidoptera: Gracillariidae), which was released in 1996. The latter agent attacks young leaves only and has no discernible impact on mature trees. The number of *L. laevigatum* buds on mature trees that are galled by *D. strobila* was monitored from 1994 until 2008. Initially the prognosis for biological control by *D. strobila* was extremely promising. However, the numbers of galls then declined sharply at most of the sites, on average to less than half of their previous peak levels. Gall-midge mortality, induced by native parasitoids, was very low initially, and, some years later, peaked at an average of only about 8 %. In 2004, predatory mites, mostly *Pyemotes* species (Trombidiformes: Pyemotidae), were discovered, killing an average of 27 % (9.8–61.3 %) of the *D. strobila* larvae and pupae in the galls, but their role in regulating populations of *D. strobila* has not been proven. A chemical exclusion experiment on seedlings showed that leaf damage by *A. thalassias* together with galling by *D. strobila* reduced the growth of young *L. laevigatum* plants by nearly 50 %, but, again, the impact of the two agents in aggregate, on mature plants, is negligible. A gall-inducing scale insect is presently under consideration as a potential agent, and there are some other possible agents that might be useful, but, overall, the prospects for biological control of *L. laevigatum* do not appear to be good.

Key words: *Aristaea thalassias*, Gracillariidae, gall midges, *Dasineura strobila*, Cecidomyiidae, predation, parasitism, gall-inducing scale.

INTRODUCTION

Over the last two centuries, a number of introduced tree species have become highly invasive throughout the wetter areas of South Africa (Shaughnessy 1980; Henderson 2001) and have been described as 'transformer' species, *i.e.* 'Plants which can as monospecies dominate or replace any canopy or sub-canopy layer of a natural or semi-natural ecosystem, thereby altering its structure, integrity and functioning' (Henderson 2001). The worst transformer species in the extraordinarily species-rich native vegetation of the Cape Floral Kingdom (the 'fynbos) (Cowling *et. al.* 1997) of the southwestern, southern and eastern Cape, are *Acacia* species (Mimosaceae) and *Hakea* species (Proteaceae) from Australia, and *Pinus* species (Pinaceae) from Europe and North America (Taylor 1969). Australian myrtle, *Leptospermum laevigatum* (Gaertn.) F.Muell. (Myrtaceae) (Fig. 1) is of lesser, but rapidly increasing importance in this biome.

Leptospermum laevigatum was introduced in the 1830s for dune stabilization, and was also used as a hedge plant (Johnson 1978; Shaughnessy 1986). It is a densely-branched, often multi-stemmed shrub or tree that grows to a height of 8 m, and flowers in spring (August–November). The fruits mature the following year and comprise semi-fleshy, flat-topped, cup-shaped capsules (about 7 mm in length and 8 mm wide) that are serotinous (remaining on the plant for two to three years). Each fruit contains 80–130 small seeds (each 1–2 mm in length) that are adapted for wind dispersal (Johnson 1978). In South Africa, the plant has a restricted and disjunct distribution along the coastal regions of the southwestern, southern and eastern Cape (Henderson 2001; Fig. 2). *Leptospermum laevigatum* is becoming more dominant as an invasive plant on sandy soils in fynbos vegetation, because it is replacing *Acacia saligna* (Labill.) H.L.Wendl. (Mimosaceae) (Gordon 1999; Morris 1999; Wood & Morris 2007), and other invasive

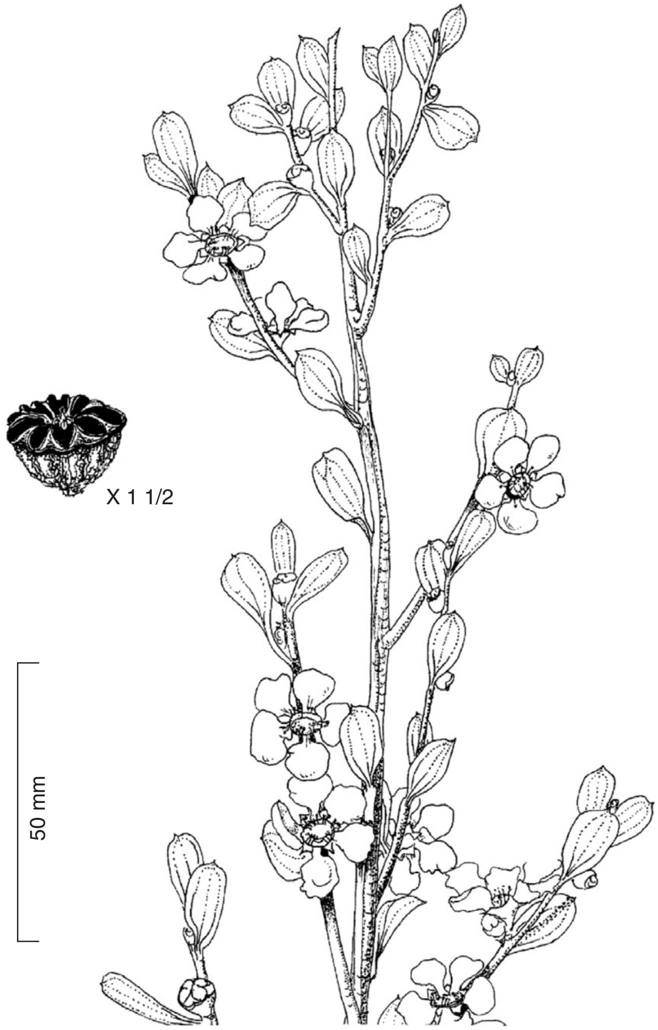

Fig. 1. *Leptospermum laevigatum.* (Drawn by R. Weber; first published in Stirton (1978), South African National Biodiversity Institute, Pretoria.)

tree species that are themselves under substantial biological control. In Australia, *L. laevigatum* has a discontinuous native distribution along the coastal regions of New South Wales, Victoria, Northern Tasmania and the Bass Straits (Burrell 1981). It has also become naturalized in Western Australia, New Zealand, California and Lana'I (Hawaii) where it is considered a problematic environmental weed (Lam 2002).

Although *L. laevigatum* plants are usually killed during a fire, the small seeds are protected in the capsules and are released soon after the fire when prolific germination of seedlings can result in dense stands of the weed. Chemical control measures are expensive and the difficulties of mechanical controls are exacerbated by coppicing of multi-stemmed plants which are less tractable to sawing and chopping.

Two insect species are currently utilized in South Africa for the biological control of *L. laevigatum*: a leaf-mining moth, *Aristaea* (*Parectopa*) *thalassias* (Meyrick) (Lepidoptera: Gracillariidae), which was released in 1996, and a recently described bud-galling midge, *Dasineura strobila* Dorchin (Diptera: Cecidomyiidae) (Dorchin & Adair 2011), which was inadvertently introduced, possibly in the mid 1980s.

The preliminary results of biological control

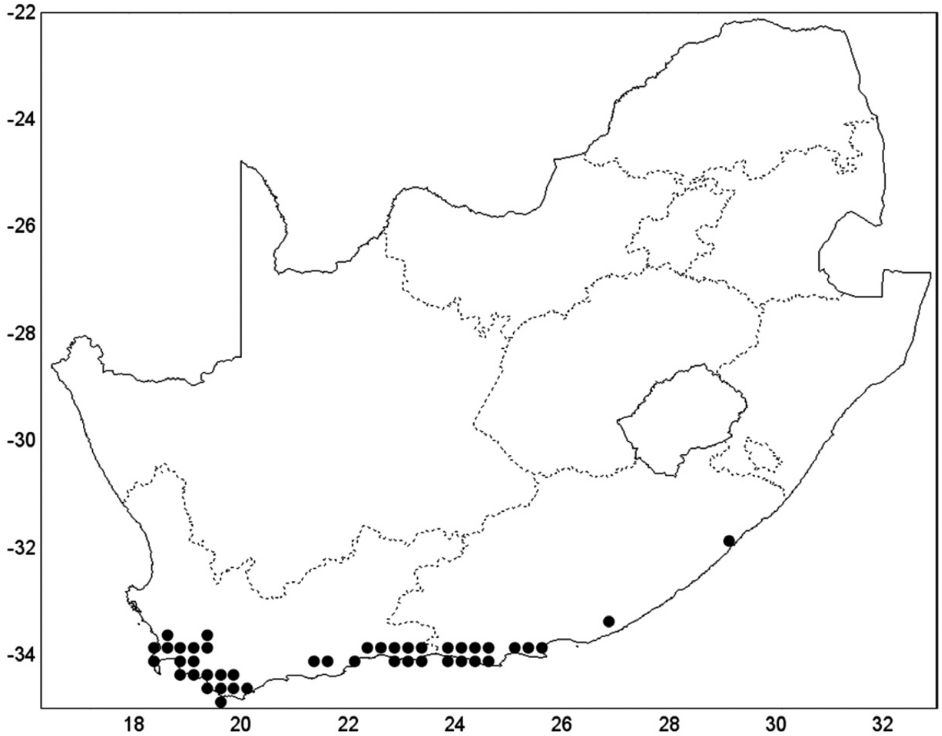

Fig. 2. Distribution of *Leptospermum laevigatum* in South Africa. (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria.)

against *L. laevigatum* in South Africa were reviewed by Gordon (1999), who provided details on the biology, release and establishment of *A. thalassias.* At that stage *D. strobila* had only just become established and little was known about it, but there was cause for optimism about this programme as a whole. The present review gives a brief update on *A. thalassias,* but the emphasis is on factors affecting the performance of *D. strobila* as a biological agent.

THE BIOLOGICAL CONTROL AGENTS DEPLOYED

Aristaea thalassias

These small moths (5 mm in length) lay their eggs on the upper and lower surfaces of new leaves. They are multivoltine and their rapid development permits 5–6 generations per year. The early-instar larvae of *A. thalassias* form thin, serpentine mines in the new leaves of the plant. As the larvae feed and grow, the mines develop into large dead blotches that cause premature leaf abscission (Gordon 1999). From 1996 to 1999, large numbers (>30 000) of the leaf-mining moths were released at 60 release sites (Gordon 1999), and in January 2000, on an additional isolated infestation of *L. laevigatum* at Umtata River mouth (31°56.718′S 2911.967′E) in KwaZulu-Natal where 4510 pupae were released. Following these releases, *A. thalassias* dispersed rapidly and has now become established on *L. laevigatum* throughout the range of the weed in South Africa (A.J. Gordon, unpubl.).

Dasineura strobila

The females of *D. strobila* lay their eggs in the reproductive and vegetative buds of the host and the developing larvae induce the buds of *L. laevigatum* to develop into small but characteristic galls. Each gall harbours, on average, six *D. strobila* larvae (range 1–24, n = 80; A.J. Gordon, unpubl.) and galling of reproductive buds precludes fruit and seed-formation. The larvae are orange in colour and their average length is 1.8 mm (range 1.4–2.0 mm, n = 20), and width 0.7 mm (range 0.5–0.8 mm, n = 20). Pupation takes place in the galls and there are three to four generations a year (A.J. Gordon, unpubl.).

D.asineura strobila was originally imported into

Table 1. Releases of the gall midge, *Dasineura strobila* on *Leptospermum laevigatum* in South Africa.

Locality	Coordinates	Year released
Port Elizabeth	34°00.103′S 25°27.572′E	1997
Bredasdorp	34°33.071′S 19°52.527′E	1997
Fish Hoek	34°08.306′S 18°25.273′E	1997
Franschhoek	33°52.339′S 19°02.422′E	1997
Simondium	33°52.161′S 18°59.295′E	1997
Bot River	34°14.114′S 19°11.100′E	1997
Houwhoek	34°12.820′S 19°09.736′E	2000
Fisherhaven	34°21.980′S 19°08.530′E	2000
Stanford	34°25.638′S 19°27.765′E	2000
Elandskloof	33°57.520′S 19°18.090′E	2000
Villiersdorp	34°01.085′S 19°13.974′E	2000
Wemmershoek	33°55.243′S 19°09.314′E	2000
Paarl	33°44.315′S 18°58.672′E	2000
Agter Paarl	33°42.438′S 18°52.913′E	2000
Great Brak River	34°03.556′S 22°12.253′E	2000
Albertina	34°12.603′S 21°32.852′E	2000
Riversdale	34°11.120′S 21°25.981′E	2000
Bot River	34°14.258′S 19°11.136′E	2000
Van Stadens Pass	33°54.549′S 25°14.325′E	2000; 2001
Humansdorp	34°00.020′S 24°45.295′E	2000; 2001
Humansdorp	34°02.708′S 24°32.193′E	2000; 2001
Knysna	34°02.089′S 23°02.734′E	2000; 2001
Sedgefield	34°00.569′S 22°46.722′E	2000; 2001
Riversdale	34°11.499′S 21°27.733′E	2000; 2001
Mossel Bay	34°03.539′S 22°12.282′E	2001

South Africa in 1984 but attempts to rear it in quarantine failed. Ten years later, in 1994, *D. strobila* was re-introduced from Australia but was then discovered to be present already on *L. laevigatum* at a few sites in the Western Cape (Gordon, 1999). From the 1994 cultures, mass-rearing in a shade house proved successful and between 1997 and 2001 *D. strobila* was distributed to and became established on all the large *L. laevigatum* infestations in South Africa (Table 1).

IMPACT OF THE AGENTS ON *L. LAEVIGATUM*

Leaf-mining by *Aristaea thalassias*

The mature leaves of *L. laevigatum* are not attacked by *A. thalassias* (Gordon 1999) and consequently, less than 10 % of the leaves on mature trees are mined. Repeated mining and abscission of young leaves on seedlings can reduce their vigour and growth, but the effects of *A. thalassias* on mature *L. laevigatum* trees are negligible (A.J. Gordon, unpubl.).

Bud-galling by *Dasineura strobila*

After mass-rearing and redistribution (Table 1), *D. strobila* established readily and, by the late 1990s and early 2000s, huge numbers of midge galls were readily visible on the *L. laevigatum* infestations and a successful outcome for biological control seemed assured. After a few years, however, the proportion of galled buds on the trees declined markedly (A.J. Gordon, unpubl.). Sustained efforts were made to determine the reasons for this, including: (i) an investigation, mostly from October 2001 – June 2004, into the levels of parasitism on *D. strobila*; and, (ii) following the discovery, in 2004, of the presence of predatory mites in *D. strobila* galls, a study was initiated to determine mortality rates inflicted by mites on *D. strobila* larvae and pupae, and to assess the implications of this for the biological control of *L. laevigatum* in South Africa.

At the start of the intensive sampling period in October 2001, the number of buds galled at three sites (Stellenbosch (33°56.534′S 18°50.088′E), Kleinmond (34°17.393′S 19°08.440′E) and

Sedgefield (34°00.372′S 22°46.437′E) was low (between 0 and 10 galls/branch) but at Port Elizabeth (34°00.125′S 25°27.552′E) and at Highlands (34°16.464′S; 19°05.300′E) the numbers of galls peaked at 35 galls/branch and 29 galls/branch, respectively. Subsequently, the number of galls recorded between February 2002 and June 2004 at all five sites remained relatively low at 20 or fewer galls/branch: the average number of vegetative and reproductive buds available for galling during these mid-winter months at the study sites varied between 30 and 50 buds/branch.

From the start of the observations on levels of *D. strobila* galling on *L. laevigatum,* in the late 1990s, parasitoid exit holes in the galls were rarely encountered. By 2004, however, the average numbers of parasitoids recorded in the larvae and pupae of *D. strobila*, at nine sample sites, was about 8.2 % (1.7–25.7 %).

In February 2004 it also became apparent that *D. strobila* larvae and pupae within the galls on *L. laevigatum* were being subjected to attacks by predatory mites, mostly *Pyemotes* species (Trombidiformes: Pyemotidae). At each of nine sites in the Western Cape, 60 galls were randomly selected and dissected. There was a positive relationship between the percentage of *D. strobila* galls that contained predatory mites and the percentage mortality of *D. strobila* larvae and pupae in the galls ($y = 0.5617x + 11.033$; $R^2 = 0.7577$, $P = 0.002$). As could be expected, there was a negative relationship between the percentage of gall-midge larvae and pupae killed by predatory mites and the percentage of adult gall-midges that eventually emerged from the galls. The average level of mortality inflicted by predatory mites on the larvae and pupae of *D. strobila* in their galls on *L. laevigatum* was 26.6 % (9.8–61.3 %) (A.J. Gordon, unpubl.).

At least 12 species of mites have been recorded to date, either in, or on the galls of *D. strobila* on *L. laevigatum* (E.A. Ueckermann, pers. comm.): most have no impacts on the biological control agent because they are either scavengers, fungivores, or phytophagous. However, five of the recorded mite species are predacious, namely *Pyemotes ventricosus* (Newport) (Pyemotidae), an itch mite, which was by far the most abundant of the mites encountered; *Tydeus grabouwi* (Meyer & Ryke) (Tydeidae); *Meyerius latus* (van der Merwe) (Phytoseiidae); *Typhlodromips swellendamensis* (Ueckermann & Loots) (Phytoseiidae); and *Tydeus spathulatus* (Oudemans) (Tydeidae) (E.A. Ueckermann, pers. comm.).

The poor performance of *D. strobila* on *L. laevigatum* is probably attributable to a number of biotic and abiotic factors. Although parasitism and predatory mites are certainly destroying a large number of larvae and pupae in the galls, life-table studies are required to demonstrate the details of these interactions and to determine, in particular, whether the mite-induced mortalities are density dependent, which is a prerequisite for any definitive assertion that *D. strobila* populations are regulated by mite predation (and/or parasitism).

Aristaea thalassias in combination with *Dasineura strobila*

The impact of *A. thalassias,* together with *Dasineura strobila*, on seedling growth of *L. laevigatum* was investigated by means of a chemical exclusion experiment conducted at Bredasdorp (34°33.168′S; 19°53.055′E) from October 2000 to May 2002. The growth (number of leaves added during the course of the experiment) and the height of the seedlings, was about 50 % lower in the unsprayed plots, where the biological control agents had been present. The low leaf-count and reduced growth of untreated seedlings suggests that they were unable to compensate for the combined attacks of the insect agents.

POTENTIAL BIOLOGICAL CONTROL AGENTS

Although *L. laevigatum* in Australia does not have a rich or abundant phytophagous fauna (Andersen & New 1987; Andersen 1989; A.J. Gordon, pers. obs.) from which to select prospective biological control agents, there are a few other species of gall formers on *L. laevigatum* in Australia – some cecidomyiid flies (Cecidomyiidae) and a gall fly *Fergusonina* sp. (Fergusoninidae) (Neser 1984) – that have potential. The gall fly *Fergusonina* sp., was introduced into quarantine in 1983, 1993 and in 1994 but was never successfully cultured (Gordon 1999). There are also a number of seed-destroying insects on *L. laevigatum* in Australia (Andersen & New 1987; Andersen 1989) that could perhaps be used as biological control agents in South Africa.

The most promising of the potential agents is a gall-inducing scale-insect species, *Callococcus leptospermi* (Maskell) (Homoptera: Coccoidea) that is known to cause spectacular die-back of *L. laevi-*

Table 2. Numbers of *Callococcus leptospermi* galls imported into South Africa from Australia between 2008 and 2010.

Date	Locality	Coordinates	No. collected
February 2008	Port Botany Bay, New South Wales	33°57.590′S 151°12.339′E	181
	Otford, New South Wales	34°13.395′S 150°59.871′E	58
	Philip Island, Victoria	34°30.987′S 145°21.586′E	156
January 2009	Otford, New South Wales	34°13.395′S 150°59.871′E	121
	Philip Island, Victoria	34°32.557′S 145°41.041′E	291
February 2010	Philip Island, Victoria	34°32.557′S 145°41.041′E	287
	Otford, New South Wales	34°13.395′S 150°59.871′E	52
March 2010	North Head, New South Wales	33°49.222′S 151°17.222′E	908

gatum branches and trees in Australia (A.J. Gordon, pers. obs.). First-instar females feed on maternal gall-tissue and disperse from the gall as newly moulted second-instar nymphs that migrate to new galling sites on the branch (Coles *et. al.* 1988). *Callococcus leptospermi* was collected in Australia from 2008 to 2010 and brought into quarantine in South Africa, but no offspring were obtained due to high levels of parasitism. The galls collected varied in size from 6-25 mm in length and from 6-13 mm in depth. Although this insect is relatively scarce in New South Wales and Victoria, approximately 2054 galls were collected (Table 2). *Callococcus leptospermi* could be sufficiently host specific for use in South Africa because: (i) all the gallicolous coccoid species that induce covering-galls, like those of *C. leptospermi,* are restricted to one host family and, typically a single host genus; (ii) about 80 % of Australian eriococcid species occur on myrtaceous hosts only; and (iii) more than 80 % of eriococcid gall-inducers, worldwide, occur only in Australia.

There is another gall-inducing scale (*Callococcus* sp.) (Homoptera: Coccoidea) which occurs on *Leptospermum* sp., previously known as *L. juniperinum* (Sm.) Domin., in South Australia, which also may have potential for use in South Africa.

DISCUSSION AND CONCLUSIONS

Since 1950, cecidomyiid gall midges have been used as biological control agents against ten species of invasive alien plants in several parts of the world. In two previous cases, in Hawaii (in 1965) and in South Africa (in 1972), the gall midge *Zeuxidiplosis giardi* (Kieffer) (Diptera: Cecidomyiidae) successfully supplemented the actions of other biological control agents used against *Hypericum perforatum* L. (Clusiaceae) (Julien & Griffiths 1998; Gordon & Kluge 1991). Generally however, the history of success with cecidomyiids as biological control agents has been very poor. Often they have failed to establish, or their impact has been trivial (or more often has not been fully evaluated) (Julien & Griffiths 1998; Muniappan & McFadyen 2005). Although it is well documented that parasitism and predation can frustrate biological control attempts against invasive plants in general (Goeden & Louda 1976), cecidomyiids have gained a particular notoriety among biological control practitioners for their 'boom' and then 'bust' characteristics: that is to say, initially, after release, their populations increase rapidly but then decrease, often equally rapidly, ostensibly because of attacks by native parasitoids (Hymenoptera) or, more rarely, by native predators (Julien & Griffiths 1998; Muniappan & McFadyen 2005). Although extremely promising, it is too early to predict the longer-term outcome of the most recent (in 2001 and 2002) releases of two other cecidomyiid gall-midge species in South Africa, *Dasineura dielsi* Rübsaamen and *Dasineura rubiformis* Kolesik (Diptera: Cecidomyiidae) on *Acacia cyclops* A.Cunn. ex G.Don, and on *A. mearnsii* De Wild. (Mimosaceae), respectively (Impson *et al.* 2009).

For more than 25 years, South African entomologists have been trying to achieve an acceptable level of biological control of *L. laevigatum,* thus far with little success. In combination, and at some sites, *A. thalassias* and *D. strobila* may contribute to a reduction in the growth and vigour of *L. laevigatum* seedlings, but the overall suppression of the weed through these two species of biological control agents is negligible. Felling and removal of adult plants is fairly easy but seedlings and smaller plants are problematic since they are difficult to locate and to distinguish from other small plants in the fynbos vegetation. There is no doubt that populations of *L. laevigatum* are rapidly increasing and replacing other invasive tree species in the fynbos that have been or are being brought under biological control (Gordon 1999). This adds urgency to, and complicates future management strategies against *L. laevigatum* populations, and it is moot whether or not South Africa should persist with the introduction of new agents, or abandon attempts at biological control of *L. laevigatum* altogether.

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