Natural impact of the flea-beetle, *Longitarsus* sp., on *Heliotropium amplexicaule* in Argentina and its potential for use as a biological control agent in Australia

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Summary

*Heliotropium amplexicaule* (Boraginaceae) was introduced into Australia from South America over 100 years ago and has since become a serious weed in summer pastures. In 1998, CSIRO started a classical biological control project against *H. amplexicaule* to search for and evaluate natural enemies in its native range in Argentina. This study quantifies the impact of one such natural enemy, the flea-beetle *Longitarsus* sp. (Coleoptera: Chrysomelidae), on *H. amplexicaule*, to determine its potential for control of the weed in Australia. *Longitarsus* sp. adults chewed small feeding holes in the leaves of *H. amplexicaule* plants, leading to necrosis and death of the remaining leaf tissue. Feeding started from the basal leaves, with new leaves attacked as they developed on the growing plants. Unattacked control plants achieved maximum size by early March. The mean growth of attacked plants at the two attacked sites was only 54 and 48%, respectively, of that shown by these control plants. Moreover, these plants showed greatly reduced vigour and half of them had died by mid-March at one site and mid-May at the other. There was no plant mortality at the control site. Finally, control plants produced significantly larger quantities of flowers and seed over a longer period than did attacked plants. The overall impact of *Longitarsus* sp. is due to a combination of adult feeding on leaf tissue and larval feeding on the root system. The study also demonstrates a synergism between the flea-beetle and pathogenic micro-organisms causing subsequent leaf necrosis. Such impact on plant survival and reproductive potential suggests that *Longitarsus* sp. could be a good biological control agent for *H. amplexicaule* in Australia, provided it demonstrates sufficient host specificity.

Keywords: biological control, *Heliotropium amplexicaule*, impact assessment, *Longitarsus* sp.

Introduction

Blue heliotrope, *Heliotropium amplexicaule* (Boraginaceae) is a deep-rooted, semi-prostrate, perennial herb, native to South America in northern and central Argentina, southern Bolivia, Uruguay and the extreme south of Brazil (Johnston 1928). It was introduced into Australia as an ornamental plant over one hundred years ago, and has expanded its range considerably during the past four decades. There are now widespread infestations in south-eastern Queensland and northern New South Wales, with isolated populations in South Australia (Parsons & Cuthberton 1992). Blue heliotrope can compete successfully in agricultural systems with desirable crops and pasture species and cause a decline in livestock performance as a result of toxicity from the pyrrolizidine alkaloids it contains (Glover & Ketterer 1987). Its increasing rate of spread and difficulties in controlling it by conventional herbicides, make *H. amplexicaule* a serious threat to Australian agriculture.

In Argentina, blue heliotrope is a coloniser of recently disturbed areas, but populations either do not persist or remain at low densities. Survey work suggests that natural enemy attack reduces the plant’s capacity to
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Competition with later successional vegetation during the same season, resulting in *H. amplexicaule* being much shorter-lived in its native range than in Australia (Briese & Zapater 2001).

*H. amplexicaule* has a perennial life-cycle. Plants produce several new semi-prostrate branching shoots from a central root-crown in spring. These continue to grow throughout spring and summer, if rainfall is sufficient, and may produce several flowering flushes until autumn. The roots grow and thicken during this growth period, storing up nutrient reserves. As temperatures fall in late autumn, the shoots die back and the plants pass winter as an extensive root system, before using nutrient reserves to reseed in the following spring and repeat the cycle. *H. amplexicaule* may reproduce either from seed or vegetatively from roots just below the surface. Based primarily on the life-cycle of *H. amplexicaule*, Briese & Zapater (2002) described a two-pronged strategy for the biological control of this weed in Australia, in which they recommended the use of complementary agents to attack the above-ground biomass of the plant (photosynthetic tissue) and the root system (nutrient reserves), in order to reduce plant longevity, regrowth and reproduction, and thus render it less competitive with more desirable pasture species.

Surveys in the native range of *H. amplexicaule* in Argentina had identified four insect species with potential for biological control: the leaf-feeding beetle, *Deuterocampta quadrijuga* (Coleoptera: Chrysomelidae); the flea beetle, *Longitarsus* sp. (Coleoptera: Chrysomelidae), which feeds on leaves as an adult and roots as a larva; the bug, *Dictyla* sp. (Hemiptera: Tingidae), which sucks saps from the cells of leaves, killing them; and the thrips, *Haplothrips heliotropica* (Thysanoptera: Phlaeothripidae), whose feeding causes deformation of leaves and buds (Briese & Zapater 2001, 2002). Additionally, an open-field experiment conducted in Argentina showed that the four insects had very restricted host ranges and might satisfy more quarantine clearance of a candidate agent, it is essential to determine whether it has the potential to cause damage that could affect the population dynamics of the target weed in its introduced range. This paper therefore describes studies on the impact of naturally occurring populations of *Longitarsus* sp. on *H. amplexicaule* plants in its native range in Argentina, designed to determine whether it had the potential to fulfill a complementary role to *D. quadrijuga* in the biological control of the weed.

**Materials and methods**

**The experimental area**

Experimental plots were set up on abandoned roadside strips of land containing localised populations of *H. amplexicaule* at km 49 (hereafter named as site 49), km 50 (site 50) and km 51 (site 51) of Route 188, near Pergamino in Buenos Aires province, Argentina. Latitudes and longitudes of these three sites were 33°43′56″S by 60°26′15″W, 33°44′16″S by 60°26′23″W and 33°44′29″S by 60°26′38″W, respectively. Most plants at site 51 and some at site 50 had germinated the previous year in areas overgrazed by horses, while those at site 49 had germinated during the current year after a winter fire. The dominant vegetation in sites 50 and 51 was the invasive grass, *Cynodon dactylon*, while site 49 had a dense mixed grass sward. Plants from site 51 had been observed to be in good condition during the season before this study.

**The studies**

At each site, 20 plants of *H. amplexicaule* were individually identified with numbered plastic tags and visited fortnightly on 11 occasions from December 1999 to June 2000; on December 17, January 3 and 15, February 1 and 22, March 7 and 23, April 6 and 25, May 18 and June 15. At two sites (49 and 51) all plants were maintained under natural conditions to evaluate *Longitarsus* sp. damage while, at the third site (50), control plants were treated at each visit with a systemic insecticide to prevent insect attack and provide a baseline for quantifying the impact of the flea-beetle. Surveys ceased once the aerial parts of *H. amplexicaule* plants had senesced with the approach of winter.

*Longitarsus* sp. populations were estimated by counting adults on all marked plants and calculating the mean density per plant for each site and date. It should be noted that, since the *Longitarsus* sp. females lay their eggs directly onto the roots in the soil and larvae feed underground on the hair roots of *H. amplexicaule*, it was impossible to sample either eggs or larvae non-
Results

Longitarsus sp. population density

Longitarsus sp. adult population densities at the three sites during the season are shown in Figure 1. The population at site 49 fluctuated in December and January, peaked rapidly in late February–March and declined to very low levels from April on. The population at site 51 was lower than at site 49 and showed a slightly earlier, less pronounced and less persistent peak in population in early February before declining to very low levels from April on. The population at site 51 was important. As indicated earlier, although Longitarsus sp. adults are highly mobile and frequently jump to neighbouring plants when disturbed (Briese et al. 2002). At the study sites, adults were observed on other plant species within the neighbourhood of a H. amplexicaule plant. Some feeding holes were found on only one other species, Convolvulus sp. (Convolvulaceae), between February 22 and March 23, but only on plants within a radius of 0.80 m. Further away, Convolvulus sp., although present in large numbers was never seen to be fed on or even visited by Longitarsus sp. Interestingly, no Longitarsus sp. adults were observed on plants of the more closely related Echium plantagineum (in the same family Boraginaceae as H. amplexicaule) either close to or far from H. amplexicaule.

Feeding damage and leaf necrosis

In spite of the apparently low average numbers of adults observed per plant, the resulting feeding damage was important. As indicated earlier, although Longitarsus sp. adults produced only small shot-holes in the H. amplexicaule leaves when feeding, necrotic lesions rapidly spread out from feeding holes causing more extensive leaf damage and death (Fig. 2). In contrast, unattacked leaves exhibited low levels of necrosis as they aged. As expected, the larger population of Longitarsus adults at site 51 resulted in heavier feeding damage to the H. amplexicaule leaves, reaching a mean of 18 holes per leaf, compared with six holes per leaf at site 51 (Fig. 3). Feeding started from the basal leaves (lower nodes) with newer leaves attacked along the growing shoot as they developed and the more basal ones died (Fig. 3). As the season progressed, leaves on the higher nodes successively became necrotic following the pattern of Longitarsus feeding (Fig. 4). There was a low, though significant correlation between the number of feeding holes per node and the extent of subsequent leaf tissue necrosis per node; Spearman coefficient of rank correlations ($r_s$) being 0.138 ($P<0.01$) in site 49 and 0.170 ($P<0.01$) in site 51.

destructively in vivo. The feeding damage produced by adults, which chew small feeding holes in the leaves, was estimated by randomly selecting three stems on each plant and dividing them into 10 nodes, ranging from 1 (the oldest) to 10 (the youngest) leaves. A leaf from each node was then selected and the number of flea-beetle feeding holes in it counted. The amount of leaf tissue lost due to necrosis around the feeding holes was rated on a scale of 0–4; where 0 = no leaf necrosis, 1 = < 10% necrosis, 2 = 10–50% necrosis, 3 = 50–90% necrosis and 4 = > 90% necrosis. Leaf necrosis was a direct consequence of the Longitarsus sp. feeding holes, which facilitated infection of surrounding plant tissue by saprophagous plant pathogens.

Overall plant size was estimated by measuring the widest cross-section ($D_1$) of an individual H. amplexicaule plants and the cross-section at 90° to this ($D_2$) and calculating plant area using the formula: Area = $\frac{(D_1 + D_2)/4}$. Plant vigour was rated visually for each tagged plant as either 0 (in poor condition), 1 (intermediate) or 2 (vigorous). Dead plants were counted separately. These plant data were collected for each tagged plant as either 0 (in poor condition), 1 (intermediate) or 2 (vigorous). Dead plants were counted separately. These plant data were collected from each of the 11 sample dates to determine plant growth and changes in condition. Plant reproduction was estimated by counting the numbers of cymes and classing them either as flowering, if 10–100% of the buds were in flower, green (if more than 90% of flowers had progressed to the green fruit capsule stage, and mature (if cymes contained ripe fruit capsules). Counts were made on February 22 and April 25 at the three sites. The earlier date corresponded to the maturity of earlier flowering flushes, while the latter date corresponded to the maturity of flowers produced just prior to plant senescence.
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Figure 2. Progressive development of necrotic lesions from *Longitarsus* sp. feeding holes in leaves of *Heliotropium amplexicaule*.

Figure 3. *Longitarsus* sp. feeding damage on the leaves of *Heliotropium amplexicaule* plants at sites 49 and 51, near Pergamino, Argentina.

Figure 4. Progressive development of necrotic lesions in the leaves of *Heliotropium amplexicaule* following feeding damage by *Longitarsus* sp. Site 50 shows natural senescence of leaves from unattacked plants (node 1 leaves are closest to the base and node 10 at the growing tips of shoots).
At the unattacked control site 50, the oldest basal leaves began to senesce in late February with a progression of senescence of leaves along the shoot. At the last sample date on June 15, the youngest tip leaves still remained healthy (Fig. 4). The condition of senescing leaves declined much more rapidly than those affected by necrotic lesions (cf. slopes of rates of necrosis between sites 50 and 49 in Figure 4). The pattern of development of necrotic lesions differed between the two sites. Interestingly, leaf necrosis was more rapid at site 51 than at site 49, despite there being less Longitarsus feeding damage. Most leaves were destroyed in the lower half of the shoot by mid January at site 51, whereas only the basal leaves showed this level of necrosis at site 49. Most leaves were very heavily necrosed by the end of April at site 51 and, apart from a dip in May due to new leaf production after heavy rains, were all dead by mid-June. Necrosis progressed more slowly at site 49 with some terminal leaves still remaining when sampling ended on June 15. Longitarsus feeding and subsequent leaf necrosis therefore led to considerable loss of photosynthetic potential and earlier senescence of H. amplexicaule plants.

Plant growth

Plants at sites 50 and 51 (870 ± 264 cm² and 816 ± 140 cm², respectively) were larger than those at site 49 (415 ± 68 cm²) at the start of sampling, confirming the observation that they were already present the previous year, whereas the population at site 49 had recently germinated. The unattacked control plants at site 50 continued to grow and increase in size until March–April, when plants gradually deteriorated as the above ground vegetation senesced. Figure 5 shows the growth and decline of H. amplexicaule plants during the sampling period. Sites 50 (control) and 49 followed a similar pattern, peaking in size during March and then declining, though control plants attained a greater maximum area (1467 ± 217 cm²) than did the attacked plants at site 49 (2846 ± 868 cm²). Attacked plants at Site 51, which exhibited earlier and more extensive necrosis than site 49, peaked in size in February (1825 ± 418 cm²), followed by a more gradual decline (Fig. 5). The mean overall plant sizes of the two attacked H. amplexicaule populations were 48% (site 51) and 54% (site 49), respectively, of that shown by the control population (Fig. 5). While plants from site 49 could have been expected to remain smaller than the unattacked control plants, due to their smaller initial size, those at site 51 would have been expected to grow similarly, suggesting that the reduced growth was due to insect attack and subsequent necrosis. Moreover, the leaves of plants at site 51 were smaller (3–5 cm) than those at sites 49 and 50 (5–6 cm), indicating reduced plant vigour.

Plant vigour and mortality

Plants attacked by Longitarsus sp. at both sites declined in overall vigour rating between February and April, whereas those at the control site remained in good condition until natural senescence of the aboveground vegetation commenced in May (Fig. 6). This decline in vigour of plants at site 51, which showed a higher rate of necrosis, preceded that at site 49 by two weeks (Fig. 6). Moreover, none of the control plants died during the sampling period, whereas 70% of plants at site 49 and 95% of plants at site 51 were dead by the end of sampling on June 15. This mortality occurred over the second half of the sampling period from March to June (Fig. 6). Longitarsus larval feeding on the roots would have contributed to this mortality, but it was not possible to partition the effects of adults and larvae. A year after the experiment all plants from these two sites were confirmed dead.
**Plant reproduction**

*H. amplexicaule* can produce several flushes of flowering in a season, commencing in late spring. At the first sampling date in February, there was no significant difference (t-test comparisons with unequal variance) between the number of flower cymes produced at the three sites (45 ± 4 at control site 50, 52 ± 6 at site 49 and 39 ± 5 at site 51). However, flowering phenology was different, as 53% of the cymes had inflorescences at the control site, compared to 16% and 11% at sites 49 and 51, where plants were subject to *Longitarsus* attack, respectively (Fig. 7). This could indicate a slowdown in new flower production at the attacked sites. When sampled in April, the number of flower cymes per plant at the control site had increased to 89 ± 19, with 9% still flowering and 38% fully mature (Fig. 7). In contrast, there was no increase in the mean number of cymes at site 49 (52 ± 8), and almost 90% of cymes were mature with no new flowers. At site 51, there had been no further production of flowering cymes since February (Fig. 7). Hence, fruit production in the second part of the growing season was greatly reduced in the two sites that had been attacked by *Longitarsus* sp.

**Discussion**

*Longitarsus* sp. showed a close synchrony with the lifecycle of *H. amplexicaule* in its native range. Adults emerged in early spring, following the onset of new shoot growth by the plant, which they used as a food source. From laboratory studies on the time for development, the flea-beetle could undergo at least two generations in the field. The sharp population peaks in February at Site 51 and during March at site 52 most likely indicated the emergence of new generation adults from larvae that had been feeding on the roots of *H. amplexicaule*. These larvae would have hatched in late spring from eggs laid by the first generation adults. Such emergence coincided with the period of maximum plant size, and the subsequent decline in flea-beetle numbers was partly due to mortality and partly to dispersal from plants that were deteriorating due to feeding damage and necrosis. *Longitarsus* sp. are the most mobile of the insects found on *H. amplexicaule* and similar dispersal away from cut host-plants was observed during an open-field host choice experiment (Briese et al. 2002). Although not actually observed, the absence of adults over the winter period suggests that the beetle spends the winter, when *H. amplexicaule* foliage has senesced, in the soil as either larvae or pupae laid by the second generation.

It is instructive to compare the response of *H. amplexicaule* plants to different types of damage. *H. amplexicaule* has an extensive root system that acts as a nutrient reserve. When subject to instantaneous mechanical defoliation by cutting shoots, the reserves enable these to be rapidly replaced by vigorously growing new shoots from the central meristem (M.Z., pers. obs.). Insect defoliation alters this response. The leaf-feeding beetle, *D. quadrijuga*, already released in Australia for control of the weed, is much larger than *Longitarsus* sp. and feeds on foliage as both adult and larva. It can completely defoliate plants over a period of several days to a few weeks, but tends to leave much of the stem tissue intact. Plants respond similarly to mechanical defoliation, but more slowly, taking several weeks to produce new shoots, which tend to be shorter than those reshooting following rapid mechanical defoliation (Briese & Zapater 2001). This is probably because nutrients are still being directed towards the shoots as they are defoliated, which leads to depletion of root reserves and a consequent reduced regrowth once defoliation ceases. Plants are weakened, but do not die.
In the case of *Longitarsus* sp., the degree of defoliation is much less than for *D. quadrijuga*, but extends over a longer period of several months and, coupled with the synergistic damage due to leaf necrosis, can also eventually lead to total defoliation of the plant. Depletion of root reserves is therefore likely to be greater as nutrients are directed towards the attacked shoots for a longer period. Critically though in the case of *Longitarsus* sp., defoliation is coupled with the destruction of feeder roots by larvae in the soil and a subsequent reduction in nutrient and water uptake. This destruction of feeder roots by larvae in the soil and a depletion of root reserves is therefore likely to be much less than for the attacked plants wilted in mid-summer, whereas unattacked control plants and neighbouring plants of other species did not, suggesting that they were suffering greater water stress under the same conditions.

Damage by *Longitarsus* sp. appears to be cumulative in its effect. With a season, this could be seen by the measurements of plant reproductive effort, where flea-beetle damage had little impact on the early production of flowers and fruit, but greatly reduced subsequent reproductive success. There is also a suggestion that damage can be cumulative across seasons, as there was a greater reduction in plant vigour and higher mortality of plants at site 51, which were known to have been present and subject to *Longitarsus* sp. attack during the previous year. Such cumulative impact would be beneficial in Australia, where *H. amplexicaule* is considered an aggressive competitor in summer pastures and where there are presently no natural enemies of significance (D.B., pers. obs.). In its native range, *H. amplexicaule* is a coloniser of disturbed habitats (e.g. earth movement, burning, overgrazing) but populations do not persist for many years and tend to be replaced by other vegetation until the next disturbance (M.Z., pers. obs.). From the results of this study, *Longitarsus* sp. can play an important role in driving this process.

This study also demonstrates a synergism between two types of organism leading to more effective biocontrol. Interestingly, the degree of necrosis can be similar, even when there is a substantial difference in the original feeding damage (cf. sites 49 and 51). The extent and rate of development of necrotic lesions may therefore depend on other factors such as humidity, temperature and other plant stressors. While the pathogenic organism causing the necrosis was not identified, it was noted that *H. amplexicaule* plants reared in quarantine conditions did not exhibit necrosis around *Longitarsus* feeding holes (A. Walker, pers. obs.), suggesting the absence of a causal agent. The presence or not of effective saprophytes in the field in the country of introduction could therefore have an important impact on biological control success.

Overall, the results obtained on the impact of *Longitarsus* sp. on plant vigour, survival and reproductive potential suggests that it could be a good biological control agent for *H. amplexicaule* in Australia. The observation that it fed on one non-target species gives cause for some concern, though this appears to be due to short-term “overflow” feeding in the neighbourhood of the main host, *H. amplexicaule*, at a time when flea-beetle populations were at there peak, rather than colonisation. Detailed host-specificity tests are currently being carried out at the Black Mountain Quarantine Facility, Canberra, Australia, to confirm whether it is safe to release *Longitarsus* sp. in that country.

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**References**


