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Short communication

Impact of the flower-galling midge, *Dasineura rubiformis* Kolesik, on the growth of its host plant, *Acacia mearnsii* De Wild, in South Africa

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ABSTRACT

Acacia mearnsii (black wattle), although recognised as being extremely invasive and problematic in South Africa, is cultivated as an important commercial plant in parts of the country. Following the introduction of a flower-galling midge, *Dasineura rubiformis* (Cecidomyiidae), into the Western Cape Province for biological control of *A. mearnsii*, trials to confirm that galling would not affect growth rates of the host-plant were necessitated before the midge could be more-widely distributed. Insecticide exclusion of the midge from selected branches of the plant enabled comparison of growth rates of branches with high and low levels of galling. Over fifteen months the increment in branch diameter was greater on highly galled branches than on lightly galled branches with pods. This result indicates that *D. rubiformis*, while reducing seed set to very low levels, does not negatively affect growth of its host and that the midge will have no detrimental effect on the wattle forestry industry in South Africa.

1. Introduction

Dasineura rubiformis Kolesik is the most recent of 13 taxa of biological control agents to be deployed against 10 species of invasive Australian acacias in South Africa (Impson et al., 2008, 2011). All but one of the agents are phytophagous insects that develop in the flowers or seeds of their hosts, the exception being the gall-rust fungus, *Uromycladium tepperianum* (Sacc.) McAlpine (Pucciniales: Pileolariaceae), which develops in both the reproductive and vegetative parts of its host, *Acacia saligna* (Labill.) H.L.Wendl. (Morris, 1987).

The emphasis on using flower- and seed-feeding insects for biological control of acacias has been necessitated by the need to preserve the commercially valuable assets of the plants (e.g. timber, pulp, tannin, firewood and charcoal) while trying to reduce their invasiveness by curbing seed production and dispersal (Dennill and Donnelly, 1991; Impson et al., 2009). The most important of the commercially valuable acacias is *Acacia mearnsii* (black wattle) which has been reported to form a forestry industry worth more than US $552 million (de Wit et al., 2001), predominantly for the production of wood chips, pulp and tanning extracts, among other products (Feely, 2012). There are currently 128 000 ha of commercial wattle in South Africa with ownership comprising: two corporate growers (30 000 ha); 600 commercial famers (94 000 ha); and 3000 small scale growers (4000 ha) (Feely, 2012).

Four of the biological control agents that have been introduced into South Africa against acacias are gall-forming insects, including two pteromalid wasps, *Trichilogaster acaciaelongifoliae* (Froggatt) on *Acacia longifolia* (Andr.) Willd., and *Trichilogaster signiventris* (Girault) on *Acacia pycnantha* Benth., and two cecidomyiid flies, *Dasineura dielsi* Rübsaamen on *Acacia cyclops* A.Cunn. ex G.Don and *D. rubiformis* on *A. mearnsii* De Wild. Although the damage caused by these agents mainly affects the reproduction of their hosts, both of the *Trichilogaster* species indirectly also cause reductions in vegetative growth, and even plant survival, by acting as 'nutrient sinks' on their hosts (Dennill, 1988; Hoffmann et al., 2002; Dorchin et al., 2006). *D. dielsi*, on the other hand, has no deleterious effect on the vegetative growth of *A. cyclops* (Moseley et al., 2009), a finding that paved the way for approval by the South African Wattle Growers' Union for the release of *D. rubiformis* against *A. mearnsii*.

D. rubiformis is a univoltine species (Kolesik et al., 2005). Adults emerge in late spring (September–December) when their host plant, *A. mearnsii*, is flowering. Females lay eggs in the open flowers, depositing several eggs in each inflorescence. The neonate larvae feed on the ovary of the flower, inducing it to expand and deform into a convoluted cylindrical structure which becomes the gall. Each gall contains one to five larvae and each larva occupies its own locule (larval chamber) within the gall. Because most of the flowers in each inflorescence are used by the larvae, spherically arranged clusters of approximately 30 galls protrude from each peduncle (Impson et al., 2008). The galls expand through summer and reach maturity in mid winter (June–July) when the third-instar larvae leave the gall and fall to the ground

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where they spin a cocoon in the leaf litter or just beneath the soil surface. After approximately two months, the larvae pupate and the life cycle is completed a few days later when adults emerge from the cocoons (Impson et al., 2009).

Following its establishment in the Western Cape Province, a proviso was made that the midge should not be intentionally redistributed to other areas of the country until trials were conducted to confirm that, like its congener *D. dielsi* on *A. cyclops*, it would have no detrimental effect on the growth rates of *A. mearnsii*. Accordingly an insecticide exclusion trial (e.g. Morin et al., 2009) was undertaken in the Stellenbosch area (Western Cape Province) to determine whether there were any signs of reduced growth rates on *A. mearnsii* trees subject to heavy galling by *D. rubiformis*.

2. Materials and methods

2.1. Sample sites

Measurements on the growth of *A. mearnsii* were made at two localities, approximately 11 km apart, in the Stellenbosch region. At one of the sites (Helderberg — 34° 2.9′S 18° 51.2′E), *D. rubiformis* had been active for at least four seasons and had reached population levels where galling was abundant and uniform on all of the trees in the area. At the other site (Libertas — 33° 57.1′S 18° 50.2′E), *D. rubiformis* was in its first season in the area and was scarce, with low levels of sparse galling on the trees. Helderberg, with high levels of galling, served as an experimental site to measure the effects of galling while Libertas, with almost no galling, served as a procedural control to determine whether the insecticide, or the application process, had any effect on plant growth, i.e. growth of insecticide-treated and untreated branches could be compared in the absence of galling.

The sites are situated in the winter-rainfall region with a mean annual rainfall of approximately 630 mm at both sites. The elevation is 350 m at Helderberg and 150 m at Libertas. At the time of the study, both sites had stands of scattered, self-seeded *A. mearnsii* trees of various sizes with an understory of predominantly bare soil and grassy patches.

2.2. Growth measurements

At both of the sites, five comparable mature trees were selected (i.e. producing flowers, 4–6 m tall and with a basal stem diameter of 25–30 cm). On each of the five trees, 10 branches of equivalent size were tagged. Five of the tagged branches were allocated for insecticide treatment, to exclude *D. rubiformis*, and five were allocated as controls, having no insecticide treatment. Insecticide treatments were applied at weekly intervals. The branch to be treated was enclosed in a funnel shaped covering of plastic sheeting (to minimise spray drift) and sprayed with a mix of 3 ml of Bulldock® (a pyrethroid, active ingredient Beta-cyfluthrin) and 0.5 ml Agral® (wetting solution) in 7.5 l of water. The control branches were similarly enclosed in plastic and sprayed with a mixture of water and the wetting solution only.

At monthly intervals from September 2008 to November 2009, the branch diameter was measured, and the number of gall and pod clusters was counted, on each of the tagged branches. To ensure that branch diameters were recorded from the same position on each sampling occasion, expandable plastic rings were placed in the internode closest to a point on the branch where the branch diameter was approximately 5 mm at the start of the experiment. Measurements were then made of the lateral diameter of the branch at the midpoint of the internode immediately distal to the plastic ring. The few inflorescences that had both galls and pods on the same peduncle were recorded as 'mixed' clusters. Counts of gall and pod clusters were discontinued after June when gall development peaks and they start to senesce.

The mean dry mass of galls and pods at different times of the year was measured by taking monthly samples (excluding February when no samples were taken) of gall clusters and pod clusters ($n \approx 50$) from *A. mearnsii* trees that were not part of the trial. These galls and pods were oven dried to a constant mass at 80 °C for five days and weighed. A further sample of 10 gall clusters and 10 pod clusters was dismantled in March and September respectively to obtain the mean number of individual galls and pods per cluster on both untreated trees and insecticide-treated trees. The mean number of individual galls and pods per cluster was multiplied by the number of gall and pod clusters per branch, respectively, to calculate the total number of galls and pods per branch at each date. The total number of galls and pods per branch was then multiplied by the corresponding mean mass of individual galls and pods, respectively, at the same date to calculate the total mass of galls and pods per branch at monthly intervals throughout the year.

2.3. Data analysis

One way ANOVA was used to compare insecticide-treated and untreated branches for: (i) gall and pod numbers at Libertas; (ii) pod masses at Helderberg; and (iii) branch diameters at both Libertas and Helderberg. To determine the earliest date at which there was a significant divergence in branch diameters between insecticide-treated and untreated branches at Libertas, *t*-tests were used. STATISTICA 7 (StatSoft, Inc., Tulsa, OK, USA. http://www.statsoft.com) was used for all statistical analyses.

3. Results and discussion

Insecticide-treatments were not entirely effective in preventing galling on branches at Libertas, where *D. rubiformis* was abundant, but there were significantly more galls on untreated branches than on insecticide-treated branches ($F_{(1,185)} = 41.77$; $P < 0.0001$). This difference was due to there being both lower numbers of gall clusters and galls per cluster on insecticide-treated branches than on untreated branches (mean ± Standard Error mature gall clusters per branch = 44.1 ± 6.2 and 75.6 ± 16.1, respectively; mean ± SE galls per cluster = 3.5 ± 0.2 and 28.0 ± 0.5, respectively). Conversely, there were significantly more pods on insecticide-treated branches than on untreated branches (mean ± SE mature pods per branch = 44.9 ± 3.2 and 2.3 ± 0.5, respectively) ($F_{(1,275)} = 260.07$; $P < 0.0001$). These differences resulted in marked divergences in the mass of galls and pods that developed on the insecticide-treated and untreated branches throughout the season (Fig. 1). The mass of galls was consistently greater on untreated branches than insecticide-treated branches while the converse was applicable to pod mass (Fig. 1). The declining trend in gall mass between January and April on the untreated trees at Libertas was due to impeded development and premature death of a portion of the immature galls due to crowding (unpublished results). This loss offset

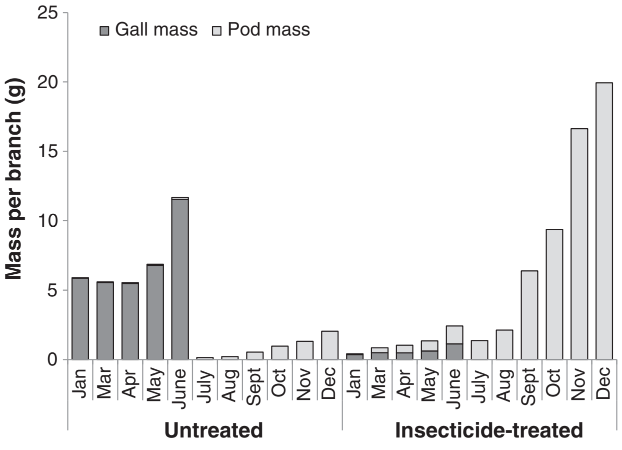

Fig. 1. Mean mass of galls (dark bars) and pods (pale bars) on untreated and insecticide-treated branches of *Acacia mearnsii* from January to December at Libertas, where *Dasineura rubiformis* was abundant.

the gain in mass that took place in surviving galls until May, when no further gall die-back occurred and there were gains in gall mass.

Galls reached maturity in July whereupon they started to senesce, while most pod development took place from August to November when the mass of pods per branch increased exponentially. The differences in phenology of galls and pods resulted in untreated branches supporting gall growth throughout autumn and early winter while insecticide treated branches predominantly supported pod growth throughout spring and early summer.

At Helderberg, where *D. rubiformis* was scarce, the pattern of increasing mass of pods was the same on both insecticide-treated and untreated branches ($F_{(1,232)} = 1.743$; $P = 0.19$), showing that the insecticide treatment had no effect on pod development. There was also no difference ($F_{(1,509)} = 0.315$; $P = 0.5749$) in the growth of insecticide-treated and untreated branches at Helderberg, showing that the insecticide treatments also had no effect on the growth of *A. mearnsii* (Fig. 2A).

At Libertas, where *D. rubiformis* was abundant, growth of insecticide-treated and untreated branches was the same for the first four months. In January the untreated branches had accumulated significantly more girth than the insecticide-treated branches ($t = 2.11$, df = 39, $P = 0.032$) (Fig. 2B). This discrepancy persisted for the duration of the trial ($F_{(1,599)} = 39.6$; $P < 0.0001$) showing that galls were no more of a burden than pods in terms of plant growth and that in fact *A. mearnsii* may grow more vigorously in the presence of *D. rubiformis* (Fig. 2B). The shorter developmental time of galls, and the greater mass of pods per branch, would account for this discrepancy with galls acting as less of a nutrient sink (see Dennill, 1988) than pods for the plants.

These results demonstrate that *D. rubiformis* will not affect the growth and therefore the productivity of *A. mearnsii* in South Africa and thus landowners who are commercially reliant on the plants need have no concerns that the insects will reduce the productivity of their crop. Indeed, the substitution of pods with galls may even enhance productivity, and thus be of benefit. Furthermore, reduced loads of seeds produced by the plants might increase the efficiency of *A. mearnsii* cultivation if there are fewer seedlings in clear-felled areas being used for new *A. mearnsii* plantations or for other types of crops. These findings not only have relevance to the situation in South Africa, but also in other developing nations where commercial and social benefits are derived from *A. mearnsii*. Finally, with reduced seed loads in the system, landowners growing *A. mearnsii*, be it commercially or at the subsistence level, will be less open to blame for spread of the plant into natural areas around cultivated woodlots.

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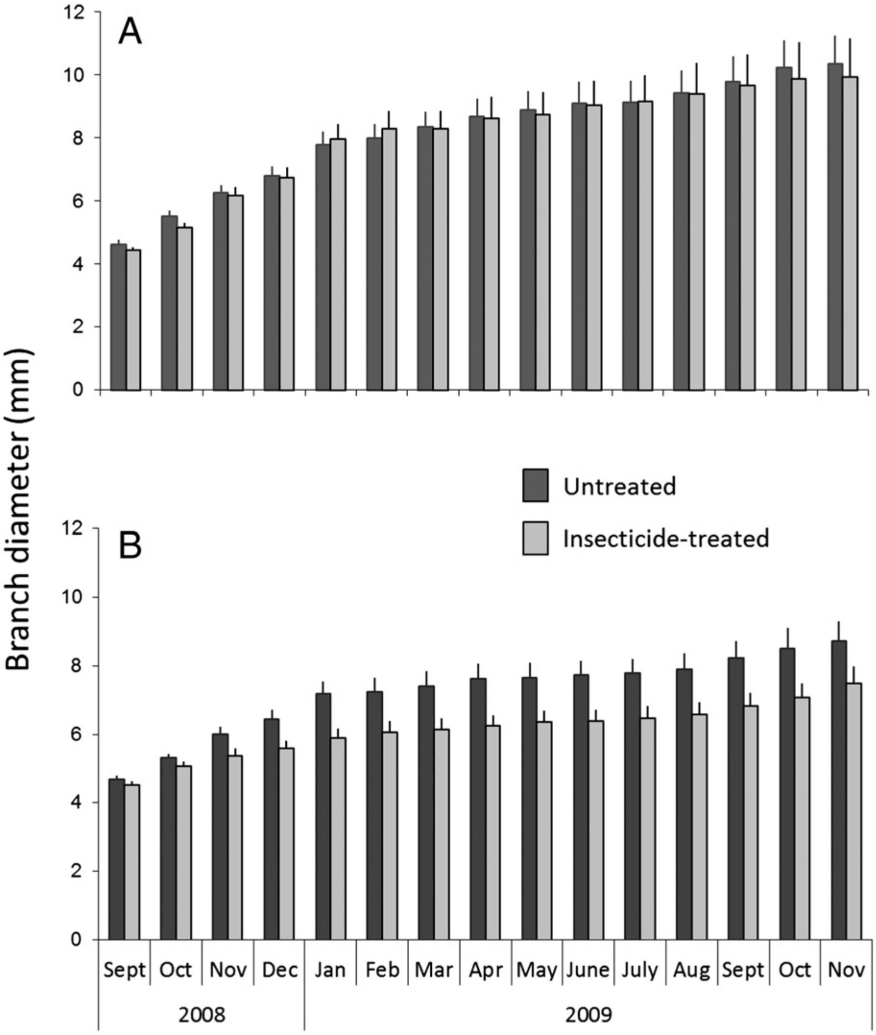

Fig. 2. Incremental growth (mean + SE diameters) of untreated (dark bars) and insecticide-treated (pale bars) branches of *Acacia mearnsii* over a period of 15 months at (A) Helderberg, where *Dasineura rubiformis* was scarce, and (B) Libertas, where *D. rubiformis* was abundant.

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