

Co-existence Between Two Leaf-Feeding Biological Control Agents of *Lantana camara* Alters Their Herbivory Under Semi-field Conditions

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Abstract

Interaction between two biological control agents released against *Lantana camara* L. (*sensu lato*) (Verbenaceae) was studied in replicated semi-field plots. Caged plants under semi-field conditions were inoculated with *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae), either alone or in combination, to investigate the extent to which co-infestation of the two agents affects the reproductive capacity and growth of their host. At the end of the trial, both single and combined attacks by the two agents had no effect on stem diameter, stem height, and canopy width. However, uncaged control plants were heavily attacked by *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae), and therefore became significantly shorter than all the caged plants in all the treatments. When confined alone, feeding damage by *O. camarae* resulted in higher reduction of fruit and flower biomass relative to that caused by *U. girardi* alone. However, when confined alone, *U. girardi* caused higher reductions in leaf density and fruit biomass than when combined with *O. camarae*. Single attack by *O. camarae* caused higher reduction in flower biomass than simultaneous attack by both agents. Above-ground biomass of all single and combined treatment plants were significantly lower than those of the caged control plants. Uncaged control plants exposed to heavy attack by *T. scrupulosa* did not produce flowers and fruits, and their above-ground biomass was significantly lower than those of caged control plants. Overall, the study showed that simultaneous attack by the two herbivores alters their herbivory, thereby affecting reproductive capacity and growth of their host.

Keywords: Biological control; Impact; Interaction; *Lantana camara*; *Ophiomyia camarae*; *Uroplata girardi*

Introduction

In classical biological control (biocontrol) of weeds, either single or multiple biocontrol agents can be released against an invasive alien plant. The release of multiple biocontrol agents against a target weed has received increased attention (McEvoy and Coombs 1999; Pearson and Callaway 2003; Denoth et al. 2002; Louda et al. 2003), with both positive (Hoffmann and Moran 1999; Anderson et al. 2000) and negative (Louda et al. 1997; Crowe and Bouchier 2006; Seastedt et al. 2007) outcomes being reported. *Lantana camara* L. (*sensu lato*) (Verbenaceae) is a good example of a weed on which over a dozen biocontrol agent species have been released and established in the introduced range (Cilliers 1987; Baars and Neser 1999; Heystek 2006).

Lantana camara, endemic to South and Central America, comprises a large complex of polyploid hybrids of widely diverse genetic composition (Urban et al. 2011). Hundreds of *L. camara* genotypes that differ in their morphological characteristics and chemical composition have been developed worldwide for horticultural purposes (Day et al. 2003). This has inadvertently improved the plant's adaptability to varying environmental conditions and its resistance to natural enemies (Gentle and Diggin 1997; Heshula and Hill 2011), adversely affecting the establishment and performance of introduced biocontrol agents (Simelane 2006; Mukwevho et al. 2017). Therefore, the rationale for releasing a suite of biocontrol agents was to cope with the climate extremes within the distribution range of lantana and adapt to varieties that are currently resistant to the established suite of agents in South Africa (Cilliers 1983; Baars and Naser 1999; Day et al. 2003; Mukwevho et al. 2017). To date, 14 biocontrol agents are fully established on *L. camara* in South Africa (Mukwevho et al. 2017; Katembo et al. 2020).

A South American leaf mining hispinid beetle *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) was among the biocontrol agents released on lantana in South Africa more than four decades ago (Cilliers 1987), and was reported to be causing significant impact on *L. camara*, particularly along the coastal regions of KwaZulu-Natal (Cilliers 1987; Baars and Heystek 2003). As biocontrol agents remained largely ineffective in the inland regions of South Africa, there was a need to release more agents to curb lantana infestations in these regions (Baars and Naser 1999; Simelane 2005). Consequently, five biological control agents were further released between 1999 and 2009 (Urban et al. 2011), and the majority of these have only established in the humid coastal regions of the country. Among these, a leaf-mining fly *Ophiomyia camararum* Spencer (Diptera: Agromyzidae) was released in 2001, and has since established and spread widely in the warm humid regions of this country and eleven other Southern and Eastern African countries.

Multiple release of different biocontrol agents against a weed species has been termed the lottery approach, suggesting that the probability of controlling the target plant would likely to increase with the number of agents released (Myers 1985; Myers et al. 1989; McEvoy and Coombs 1999). Critics have argued that the addition of more and more organisms into the system elevates the risks to non-target species (Louda et al. 2005). Releasing multiple agents could result in interspecific competition, and this has been suggested as an explanation for the failure of some organisms as biocontrol agents for weeds (Paynter and Hennecke 2001; Crowe and Bourchier 2006). Although no unintended consequences of releasing multiple *L. camara* biocontrol agents have been reported in South Africa, it is likely that competitive interaction among *L. camara* biocontrol agents exists.

Prior to *O. camararum* introduction into South Africa, Baars and Heystek (2003) had described the populations of *U. girardi* in the KwaZulu-Natal coastal region as very abundant, translating to over 50% of larva-infested leaves per plant. However, since the release of *O. camararum* and its subsequent boom in the same region, the populations of *U. girardi* have declined (Heystek 2006; April et al. 2011), indicating the possibility of a negative interaction between the two agents. Although *U. girardi* and *O. camararum* continue to co-exist and cause visible damage on leaf veins of *L. camara* along the coastal regions, population levels of *U. girardi* remain low (April et al. 2011), and that the combined effect of the two agents may be less than additive. Therefore, the study investigated the extent to which co-infestation of *U. girardi* and *O. camararum* reduced their impact under semi-field conditions. We hypothesize that, although each agent would have a negative effect on growth of *L. camara*, their combined attack would be low.

Materials and methods

Semi-field experiments were carried out in an open field at the Rietondale campus of the Agricultural Research Council (ARC) (25°43'36.7" S; 23°14'03.3" E) in Pretoria, Gauteng, South Africa. Gauteng province is situated in the highlands (Highveld) and experiences a variation in temperature, ranging from 16.1 to 30.3°C in summer with a relative humidity of 55 to 85% in the morning, 24 to 63% in the afternoon, and 32 to 78% in the evening. In winter the temperature ranges between 4.3 and 21°C. The annual rainfall average is 630.2 mm. Experiments were conducted over a period of 6 months during the summer season, from November 2006 until the end of May 2007.

Organisms used in the study

Plants used in the study were propagated from *L. camara* cuttings of variety 021 Dark Pink (021DP) cut from a mother plant in Kuswag road, Emanzimtoti [30°02'08"S; 30°53'42"E]. *Lantana camara* variety 021DP appears to be the most common variety in the coastal region of KwaZulu-Natal, and it seems to be the most preferred by both *U. girardi* and *O. camarae* (Simelane 2005). Forty-eight shoot cuttings of 7 cm length each were planted in a peat soil crate and put on a warm bed inside a glasshouse to induce rooting. Each rooted cutting was initially planted into a plastic nursery bag of 4.74 L (150 × 125 × 300 mm), with a soil mix containing two parts compost, two parts vermiculite, and one part sand before being transplanted into the experimental site. Thirty lantana plants of equal size (approximately 25 leaves per plant) were then transplanted individually into pits (30 cm × 30 cm) that were dug 3 m apart in the field at the experimental site. *Uroplata girardi* and *O. camarae* adults used to start cultures were obtained from KwaZulu-Natal Province. *Uroplata girardi* was collected either as live adults or pupae. Leaves infested with *U. girardi* and *O. camarae* pupae were collected and put into 3-L perforated containers in the lab in order to collect newly emerged adults. Moist paper towels were placed on the leaves to retain moisture, thus preventing desiccation of pupae. To mass-rear the agents, about 30 unsexed newly emerged adults of both agents were confined with potted lantana plants in cages (55 cm × 55 cm × 95 cm) in the glasshouse. Plants were watered three times a week. For both agents, leaves containing pupae were harvested, and emerging adults were collected into 3-L perforated containers. First generations of both agents were used in the experiments.

Experimental design and data collection

The thirty lantana plants that were transplanted individually at the experimental site were allowed to grow in the field uncovered for 5 weeks and were irrigated three times a week. Twenty dome-shaped cages (1.8 m × 1.8 m × 1.8 m), made of Psylla screen mesh with a sleeve in the center of the floor, were erected over the plants. Access to the cages was through a zip fastener. The experimental design consisted of two controls and three treatments arranged in a randomized block design. The first control comprised of five uncaged plants (uncaged C). The second control comprised of five caged plants (one plant per cage) and contained no insects (caged C). The first treatment comprised of five caged plants infested with 15 pairs of *U. girardi* adults per cage at the beginning of the experiment. The second treatment comprised of five caged plants infested with 15 pairs of *O. camarae* adults per cage at the beginning of the experiment. The third treatment comprised of five caged plants infested with 10 pairs of adults of each of the two (*U. girardi* and *O. camarae*) insect species per cage.

To determine the impact of the biocontrol agents in each treatment, several plant growth parameters were measured namely leaf density, stem diameter, plant length, and canopy size. Leaf density was determined by counting the number of leaves per plant in each treatment. Stem diameter was determined by measuring the base of the stem with a vernier calliper. Plant length was determined by measuring the plant height from the base to the tip of the plant. The canopy size (length \times width) was measured by a measuring tape. All the growth parameters were measured and recorded at the start of the experiment (i.e., when plants were inoculated with agents). Based on the final measure of each of the parameters of the control (caged) and treatment plants, percentage reduction of growth and biomass was calculated. All growth parameters were measured at end of the experiment. To measure biomass, five plants were excavated at the start of the trials and five plants for each control and treatment were excavated at the end of the trial. The above-ground plant parts (i.e., flowers, fruits, leaves, and branches) were separated. All the plant material was chopped, put into labelled paper bags, oven-dried at 72°C for 48 h, and weighed.

Data analysis

One-way analysis of variance (ANOVA) was performed to examine differences in the number of leaves, stem diameter, plant height, plant canopy, and plant biomass among treatments. When applicable, mean separations were done using the Tukey's test ($P < 0.05$) (Statistica vers. 13.3. 2017). To stabilize the variance, the data were square root-transformed before being subjected to parametric statistical analysis, but untransformed data are shown as mean \pm SE.

Results

Leaf density

Single and joint attack by *U. girardi* and *O. camarae* had a significant ($F = 6.12$; $df = 4$; $P < 0.05$) impact on leaf density during the 6-month period (Fig. 1). Single attack by *U. girardi* caused a significant reduction in the number of leaves per plant compared that caused by *O. camarae* alone and by both agents combined. The caged control plants had a significantly higher number of leaves compared to the uncaged control and the other treatment plants. Although *U. girardi*-attacked plants continued to be suppressed throughout the study, *O. camarae*-attacked plants and those jointly attacked by both agents appeared to recover soon after March 2007. By May 2007, leaf density on plants attacked by *U. girardi* had been reduced by 54% while those attacked by *O. camarae* alone and by both agents had been reduced by 19 and 29%, respectively. The sap-sucking tingid *Teleonemia scrupulosa* Stål (Heteroptera: Tingidae) was the only established agent that colonized and caused visible feeding damage on the uncaged plants. By May 2007, most of the uncaged plants had been severely stunted by this agent, causing 90% reductions in leaf density.

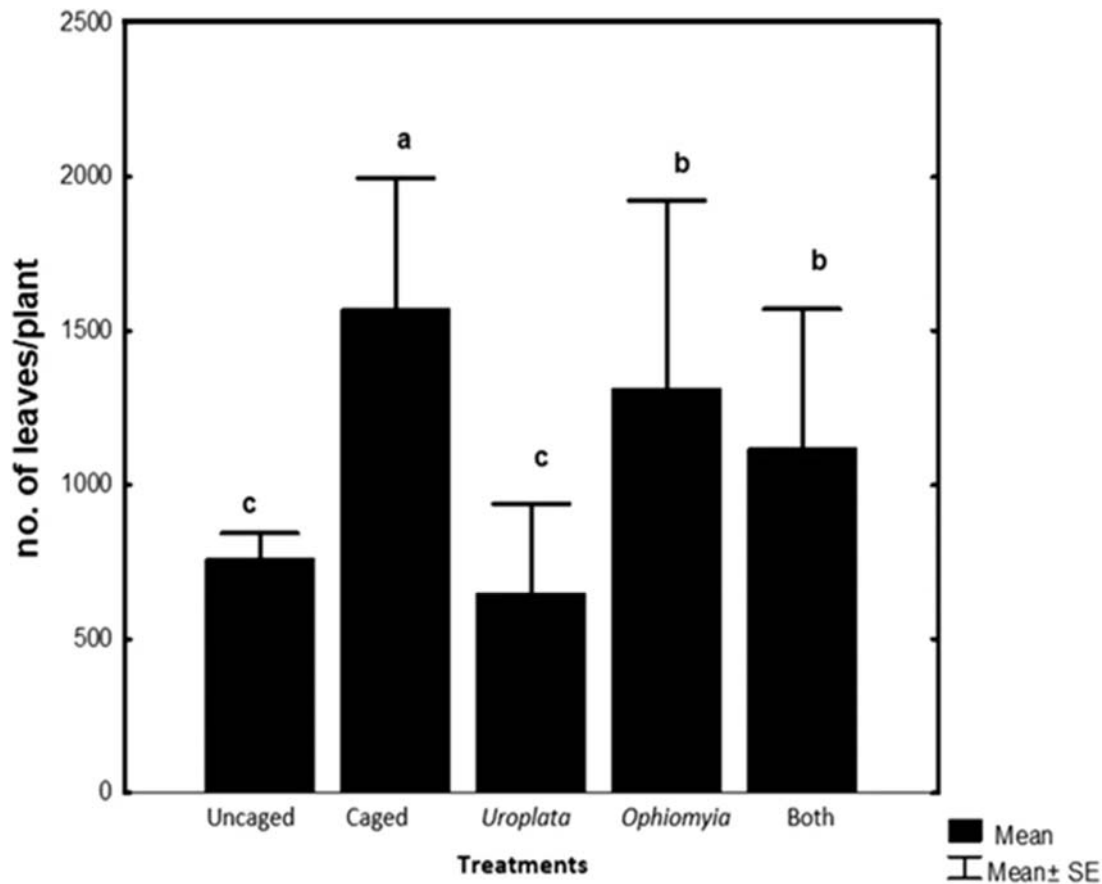


Figure 1. Number of leaves per plant (leaf density) in *Uroplata girardi*, *Ophiomyia camarae*, combined treatments, and controls. Means followed by the same letter(s) are not significantly different ($P > 0.05$; Tukey's test)

Stem diameter

Although the stems of plants that were jointly attacked by both agents were slightly thicker than those of other treatments, diameter of these stems did not differ significantly from those of other treatments and controls at the end of the trial ($F = 0.767$; $df = 4$; $P = 0.547$).

Plant height

Due to heavy attack by *T. scrupulosa*, uncaged control plants were markedly stunted, and were therefore significantly shorter than those of other treatments and caged control plants ($F = 3.904$; $df = 4$; $P = 0.017$). However, the plant height of caged control plants did not differ significantly from other treatment (Fig. 2).

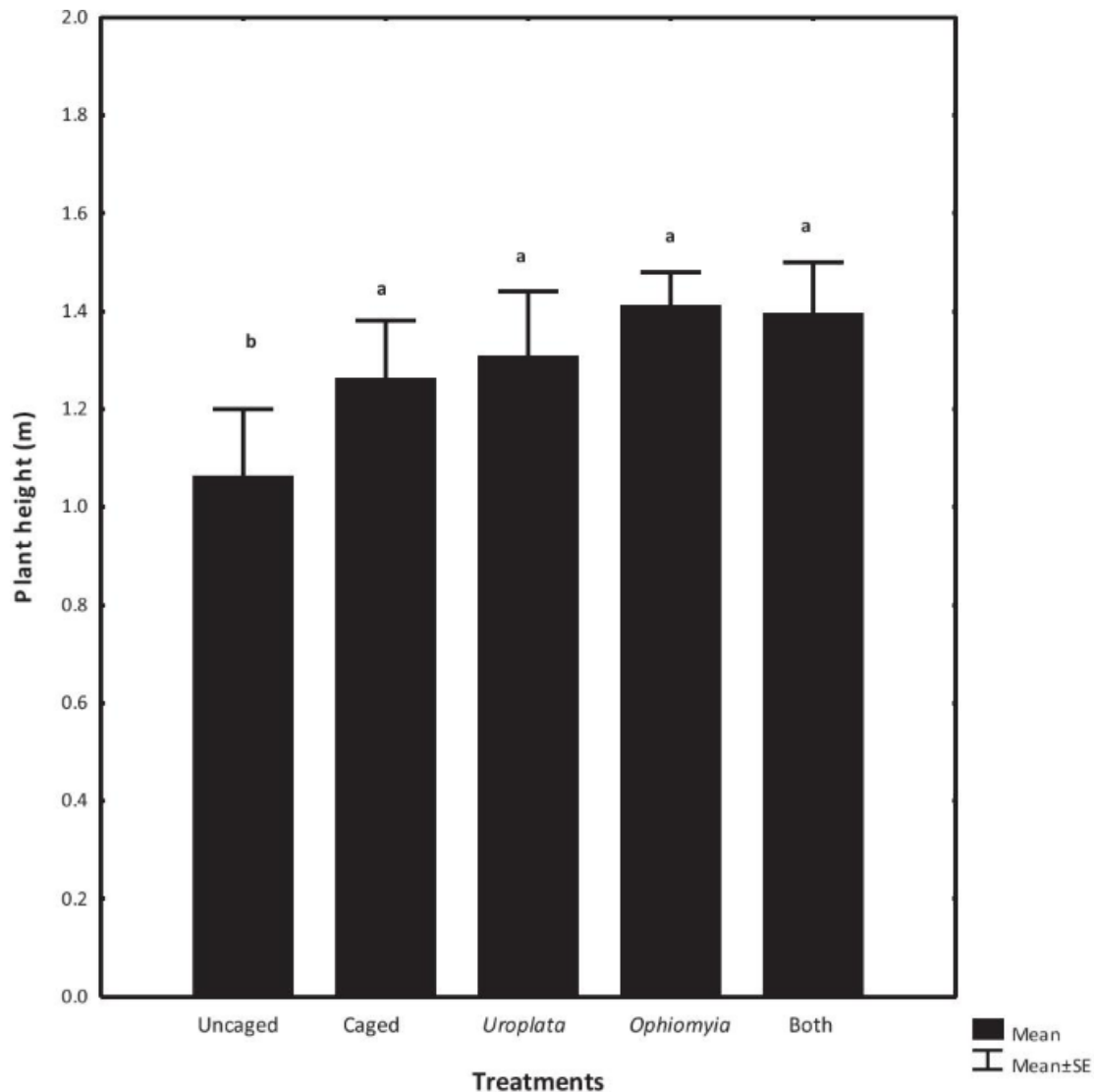


Figure 2. Final plant height of *Lantana camara* in different treatments and controls. Means compared with one-way ANOVA; those followed by the same letters are not significantly different ($P > 0.05$; Tukey's test)

Canopy size

By the end of experiment, all treatment and control plants had almost the same canopy size ($F = 1.2$; $df = 4$; $P = 0.4$). Despite being significantly shorter than other treatments (see Fig. 2), the canopy size of uncaged control plants did not differ significantly from those of other treatments by end of the trials.

Dry plant biomass

Joint and single attack by *U. girardi* and *O. camarae* had a significant impact on the biomass of lantana reproductive parts ($F = 203$; $df = 4, 45$; $P < 0.05$) (Fig. 3). Uncaged control plants did not produce flowers due to heavy attack by *T. scrupulosa*. When *U. girardi* and *O. camarae* were confined separately, they reduced flower biomass by 12 and 39%, respectively, while reducing fruit biomass by 42 and 63%, respectively. However, fruit and

flower biomass of plants that were jointly attacked by both agents were 19 and 29% lower, respectively, than caged control plants. Although the above-ground biomass of *U. girardi*-attacked was slightly lower than that of *O. camarae*- and jointly attacked plants ($F = 154.09$; $df = 4,45$; $P < 0.05$), the three treatments did not differ significantly (Fig. 4). The above-ground biomass of *U. girardi*-, *O. camarae*-, and jointly attacked plants were 52, 44, and 49% lower, respectively, than those of the control plants. The above-ground biomass of uncaged control plants were 90% lower than those of the control plants.

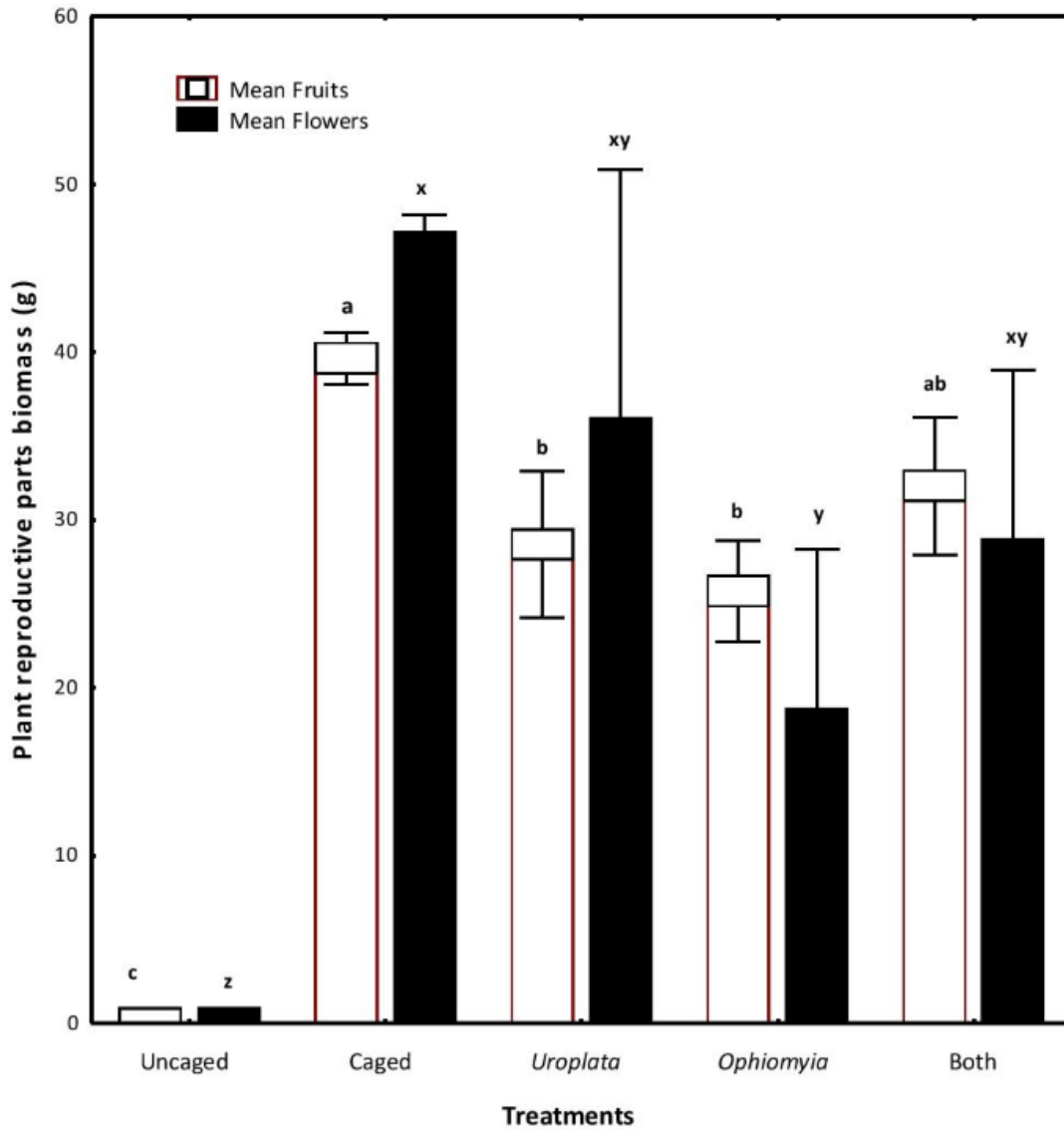


Figure 3. Dry biomass of reproductive parts (mean \pm SE) in *Uroplata girardi*, *Ophiomyia camarae*, and combined treatments and controls. Means followed by the same letter(s) are not significantly different ($P > 0.05$; Tukey's test). For fruit biomass, letters a, b, and c are used whereas letters x, y, and z are used for flower biomass

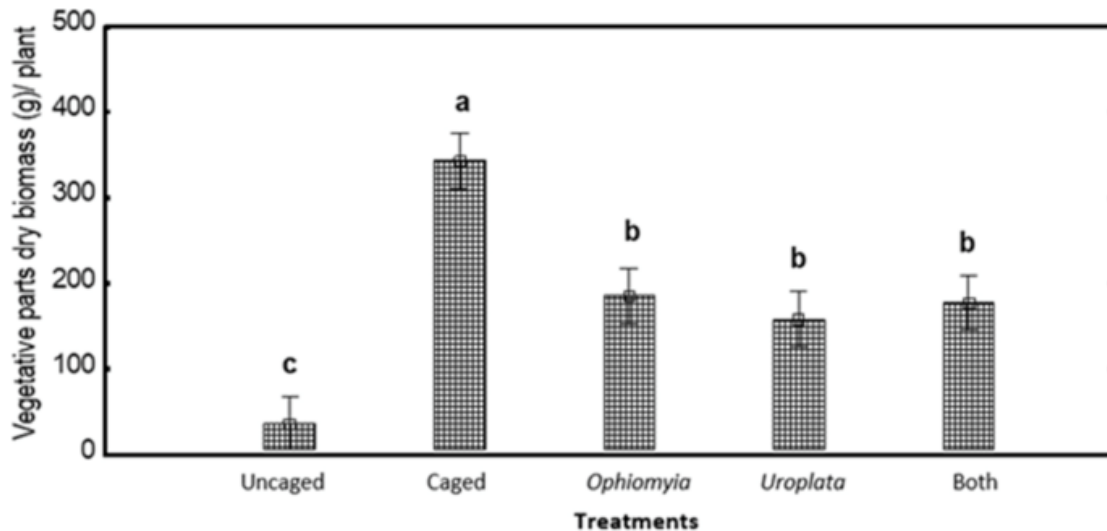


Figure 4. Above-ground dry biomass (mean±SE) in *U. girardi*, *Ophiomyia camarae*, combined treatments, and controls. Means followed by the same letter(s) are not significantly different ($P > 0.05$; Tukey's test)

Discussion

The results suggest that single attack by *U. girardi* reduced leaf density by 54% compared to 19% by *O. camarae* alone. However, the combination of *U. girardi* and *O. camarae* reduced leaf density by only 29%, which was much lower than what *U. girardi* does alone, and this is an indication of a negative interspecific interaction between the two species. However, given that *O. camarae* chooses to oviposit on clean rather than *U. girardi*-infested leaves (April et al. 2011), it is likely that under field conditions the fly would disperse from *U. girardi*-infested leaves and oviposit on cleaner ones, thus mitigating the effect of interspecific competition. This is consistent with other studies that have found that agromyzid leaf miners often prefer undamaged leaves (Faeth 1985) for oviposition and this strategy also serves as a post-fertilization investment in some insect species (Faeth 1985). Both agents had a significant effect on reproductive output of the plant when confined individually than when combined, with *U. girardi* reducing fruit biomass by 42%, *O. camarae* by 63%, and both agents by 19% relative to the control. The negative effect of *O. camarae* on flower and fruit biomass in individual treatments was remarkably similar to that reported by Simelane and Phenyne (2005). Although *U. girardi* and *O. camarae*, either individually or combined, had a significant effect on above-ground biomass (Fig. 4), they had no effect on stem diameter, plant length, and canopy size. Our study evaluated the short-term impact of the two agents, and therefore only the most vulnerable parts of the plant were likely to be affected during the study period.

Overall, inoculation with individual insect species had a greater impact on leaf density and reproductive output than both agents combined. However, the shorter pre-oviposition (2–3 days after adult emergence) for *O. camarae* (Simelane 2002) and longer pre-oviposition period (about 3 weeks after adult emergence) for *U. girardi* (Harley 1969) suggest that earlier utilization of leaves by *O. camarae* in combined treatments may have resulted in the depletion of food resource for *U. girardi* which required a longer development period. The fact that both *U. girardi* adult and larval stages feed on leaves of the same plant suggests that the fly has a better chance of surviving and completing its life cycle when confined alone, thereby intensifying damage and suppressing plant growth. Our study corroborates with that

of Crowe and Bouchier (2006) who found that, despite their co-existence, the combined attack by gall-fly *Urophora affinis* Frfld. (Diptera: Tephritidae) and seed-head weevil *Larinus minutus* Gyll. (Coleoptera: Curculionidae) Gyll. (Coleoptera: Curculionidae) on knapweed *Centaurea stobe* L. ssp. *Micranthos* (Asteraceae) was significantly less than that caused by *L. minutus* alone.

The uncaged control plants were significantly affected through loss of leaves caused by the tingid *T. scrupulosa*, resulting in stunted growth and reduced plant reproductive ability (Figs. 2, 3, and 4). Simelane and Phenye (2005) also reported that *T. scrupulosa* is widespread and causes significant effect on plant growth. In addition, other environmental factors may have played a role in the reduction of plant growth in uncaged plants. According to Raghu et al. (2006) plants experience damage from a wide variety of environmental factors (abiotic and biotic) and exhibit a diversity of responses that can be broadly classified into susceptibility and resistance. Therefore, it is likely that the exclusion trials by cages might have prevented other factors that inflict damage on the uncaged plants such as exposure to wind, sun, dryness, and raindrop intensity. Our results together with those reported by April et al. (2011) support the notion that the decline in the population density of *U. girardi* in KZN Province (Heystek 2006) is due to co-infestation by both agents which has a negative effect on *U. girardi* and a minor effect on *O. camarae* populations. April et al. (2011) found that colonization of *L. camara* by both *U. girardi* and *O. camarae* reduced the population density of *U. girardi*, with the infestation level by the beetle averaging 9% per plant when combined with *O. camarae*, compared with 29% per plant when confined alone. Similarly, field surveys in KZN indicated that *U. girardi* abundance levels ranged from 25 to 50% of infested leaves per plant prior to *O. camarae* release (Baars and Heystek 2003), and this declined to 2 to 7.5% four years after the release of the leafminer (April et al. 2011). Therefore, the reduction of impact on leaf and reproductive biomass in combined treatments suggests that simultaneous herbivory by *U. girardi* and *O. camarae* could be having a negative effect on biocontrol. Although our results did not display an additive effect of the two biocontrol agents, there is a possibility that additive interactions could still develop in field conditions due to spatial and temporal partitioning of food resources (Schoener 1974, 1982). As argued by MacNally (1983), dietary overlap does not necessarily lead to exploitative competition unless food resources are limiting, and this could partially explain why *U. girardi* and *O. camarae* continue to coexist in the field.

Nonetheless, the decline in the population of *U. girardi* in the KZN Province suggests that simultaneous herbivory by the two agents may be having a negative effect not only on the population of this beetle but also on its ability to control *L. camara*. The severe damage caused by *T. scrupulosa* on uncaged control plants seems to agree with the findings by Katembo et al. (2020) which showed that this bug and the complex of other *L. camara* biocontrol agents are enhancing control of the weed more than previously thought. While antagonistic interactions between *U. girardi* and *O. camarae* do occur, it is unlikely to diminish the combined impact of the two *L. camara* biocontrol agents.

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