

Ecological Data Key to Building Successful Biocontrol Programs: A Case Study Using *Chrysochus asclepiadeus* (Coleoptera: Chrysomelidae) Against *Vincetoxicum* spp. (Apocynaceae)

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Summary

Native from Eurasia, *Vincetoxicum rossicum* (Kleopow) Barbar Moench and *V. nigrum* (L.) Moench (Apocynaceae) invade forested landscapes of eastern North America where they prevent regeneration of native species. A third species, *Vincetoxicum hirundinaria* Medik., was also introduced but never reached invasive status. The Eurasian phytophagous beetle, *Chrysochus asclepiadeus* (Pallus) (Chrysomelidae), was studied as a potential biocontrol agent because both adults (leaves) and larvae (roots) feed preferentially on *Vincetoxicum*. In the framework of developing a biocontrol programme in North America, we asked three key questions: 1) How does the beetle disperse in its environment?; 2) How does the beetle impact plants under different natural light conditions?; and 3) How do plants react to natural or simulated herbivory? Using mark-release-recapture, we determined that most beetles were flightless and dispersed <15 m after 25 d. Under differential light conditions, beetles defoliated all three *Vincetoxicum* spp. significantly less in full sun than in partial or full shade, suggesting that herbivory was influenced by either temperature on beetle behavior, light on leaf quality, or both. *Vincetoxicum hirundinaria* was clearly able to compensate for loss of leaf tissue by increasing its dry root biomass relative to its shoot biomass reflecting a long relationship with *C. asclepiadeus*. Both *V. nigrum* and *V. rossicum* were unable to compensate for leaf tissue lost to herbivore feeding in partial and no sun and in all light conditions, respectively. Simulated herbivory reduced *V. rossicum* growth by 30% and root herbivory and plant resistance by 60%, and the combined effect of leaf and root herbivory reduced sexual reproduction of *V. rossicum*. Data on natural herbivory showed that while *V. hirundinaria* increased allocation of resources to roots, *V. nigrum* did not. If *C. asclepiadeus* was introduced for controlling *V. rossicum* in North America, reductions in plant biomass and spread would be greatest if beetles were released on forest edges at low plant densities. In the case of *V. nigrum*, beetles could be released irrespective of plant density, but populations in forest understories would be more impacted than those in full sun. For both species, multiple beetle releases would facilitate dispersion and improve biocontrol.

Introduction

Over the past three decades two Eurasian species of swallow-wort, *Vincetoxicum rossicum* (Kleopow) Barbar Moench and *Vincetoxicum nigrum* (L.) Moench. (Apocynaceae), have become invasive in northeastern North America (NA) and now are a significant threat to native biodiversity. Both species are herbaceous, perennial vines related to native North American milkweeds. Swallow-worts have been present in NA for over a century, but have started to spread only recently. *Vincetoxicum rossicum* tends to be shade-tolerant and is found further into forest understories than *V. nigrum*, which tends to be limited to habitat edge and open areas (Milbrath 2008). Invasive *Vincetoxicum* spp. impact native faunal and plant species by out-competing endangered herbaceous species and preventing natural tree species regeneration (Tewksbury et al., 2002). There is currently no effective method to regulate populations, and biological control has been suggested as one of the few options available to provide long-term control (Miller et al., 2008). From a list of potential natural enemies in Eurasia (Weed and Casagrande, 2010; Sforza, 2009), the chrysomelid beetle *Chrysochus asclepiadeus* Pallas (Col.: Chrysomelidae) was selected for study because all developmental stages feed on *Vincetoxicum* spp.: as larvae on the roots and as adults on the foliage.

In order to evaluate the potential to use a biocontrol candidate such as *C. asclepiadeus*, we initiated ecological studies to examine the impact of beetle density on herbivory (Maguire et al., 2011) in conjunction with host specificity testing (see Poster - Sforza et al., this volume). Here, we report results from three experiments that asked key questions addressing beetle dispersal and herbivory: 1) How does *Chrysochus* disperse in its environment? (Experiment 1); 2) How does it impact plants under different natural light conditions? (Experiment 2); and 3) How do plants react to natural or simulated herbivory? (Experiment 3). Specifically, the third experiment evaluated the impact of leaf and/or root herbivory on *V. rossicum* under controlled environmental conditions.

Material and Methods

Plant material

Three species of *Vincetoxicum* were tested at the USDA's European Biocontrol Laboratory in Montpellier, France (EBCL). To address the first two experimental questions (Experiments 1 and 2), *V. hirundinaria* (widespread in Eurasia but not invasive in NA) was collected from native and non-invasive field populations in Mont Ventoux, France in November 2009. For Experiment 2, *V. rossicum* was collected from invasive field populations in Wehle State Park, New York, USA in July 2009, and *V. nigrum* was collected from invasive field populations in Bear Mountain State Park, New York, USA in September 2009. *Vincetoxicum rossicum* and *V. nigrum* rootstocks were shipped to EBCL and arrived on 17 March 2010. To address the third question (Experiment 3), all *V. rossicum* were field collected in April 2010 from High Park, Toronto, Ontario, Canada.

Insect source

For Experiments 1 and 2, *Chrysochus asclepiadeus* beetles (sex ratio 1:1) were collected in June 2010 on their host plant *V. hirundinaria* from Jura, France. The beetles were stored in cardboard cylinders for transportation to EBCL and then maintained under laboratory conditions on plants of *V. hirundinaria* until use.

Experimental design, data collection and analysis

Experiment 1: Forty groups of five *V. hirundinaria* plants were out-planted in a 60-m diameter circle at EBCL every 5 m from a center point. Additional groups of plants were similarly distributed along a diagonal axis, every 5, 10, and 25 m in the same field. Three hundred beetles were marked with colored and numbered stickers (bee marking kit, Thorne ®) to identify them individually and released in the field at the centre of the circular experimental site on 12 July

2010. Beetles were counted twice daily at 7 and 9am during the 4-week field experiment.

Experiment 2: A 3x3x2 factorial experiment in an outdoor randomized complete block design was used at EBCL using light intensity, plant species, and herbivore density as the factors. The three light level treatments were: full sun, partial sun, and no sun. Full sun conditions were achieved by placing plants directly in the field while partial sun conditions were achieved by placing them under trees. No sun conditions were achieved by placing a tarp over the plants in the field 1.5m off the ground either under fully blocked sun (full) or under trees (partial). On 21 June 2010, beetles (sex ratio 1:1) were randomly placed on potted plants (three pots per block) in the field where they remained for three weeks. They were monitored every second day throughout the experiment and total dry biomass was compared between treatments with and without (controls) beetles under the three different light conditions. The Scheirer-Ray-Hare test was used for all data that did not meet assumptions of parametric statistics.

Experiment 3: In Toronto, Canada, 200 plants were divided in April 2010 into four treatments corresponding to simulated herbivory on leaves (L): 90% of each leaf cut, on roots (R): 90% of roots removed, and on both leaves and roots (RL). Fifty plants were randomly assigned to each treatment and divided into five plots of ten plants each; another 50 plants were left untreated as controls. After one month of growth, plants were measured in terms of the number of stems per pot, the number of nodes, and flowering stage. Foliage and roots were cut 21 days later, immediately following measurements on the roots. After 30 days, the plants were harvested and the number of follicles noted. Non-parametric Wilcoxon test and Tukey's HSD were used for statistical analysis.

Results

Results from each experiment illustrated the effective use of ecological approaches in developing a successful biological control program. Working indoor and outdoor under summer climatic conditions, we were able to compare different biotic and abiotic factors that are important for optimizing the release of a biocontrol agent against the target weed.

Experiment 1: Beetle dispersion

Beetle dispersal from the release point was observed on four different dates (Fig. 1). At $t+1d$, 84% of the 179 beetles were found 5 m away from the release point, with four beetles (3%) already at 15 m from the release. For the duration of the experiment, most insects dispersed no further than 15 m, and 67% (4/6) were found at that distance at $t+25d$, while 33% remained at 5 m near the circle center (initial release point). Only two beetles were ever found at over 15 m from release, including one at $t+14d$; both of these were recovered at 25 m.

Experiment 2: Impact of light on herbivory

Scheirer-Ray-Hare tests indicated that dry total mass was significantly different among species ($p<0.001$), light levels ($p<0.001$), and herbivory levels ($p<0.001$) (Fig. 2). Addition of beetles did not significantly affect dry total biomass of either *V. hirsutaria* under any light conditions, *V. nigrum* under full sun conditions, or *V. rossicum* under full or no sun conditions. Herbivory by beetles significantly lowered dry mass of *V. nigrum* under partial and no sun conditions ($p=0.002$ and $p<0.038$) (Fig. 2) and significantly lowered dry mass of *V. rossicum* under partial sun conditions ($p=0.001$) (Fig. 2), according to Wilcoxon rank sum tests. Total dry mass of *V. hirsutaria* control (untreated) plants was significantly higher in full sun than in no sun ($p=0.040$), and *V. nigrum* control plants had significantly higher total dry mass in partial than in no sun ($p=0.038$).

Experiment 3: Simulated herbivory

The number of follicles harvested was compared according to the treatment applied (Fig. 3). Control plants had the highest number of follicles (24.5 ± 1.81 follicles/plant) with RL plants having the lowest number of follicles (5.7 ± 0.70 follicles/plant). L and R plants had a similar number of follicles, with 17.3 ± 1.40 and 16.0 ± 1.52 follicles per plant, respectively. Follicle number recorded for control and RL plants was also significantly different from the other two treatment groups. Follicle density for L and R plants was not significantly different.

Discussion

The main purpose of our study was to understand the capacity for *C. asclepiadeus* to disperse in a natural environment, and its responses to climatic factors in the context of developing a biological program for its control in North America. Our results show that adult *C. asclepiadeus* had a low dispersion rate with limited flight capability. Dispersal, primarily through walking, has been observed in several Eumolpinae family members and may be due either to atrophy of wing muscles (Tourniaire et al., 2000) as in this case, or due to winglessness, as seen in some other genera of the family (Flowers, 2004). Only two adult insects were found at 25 m from their release point and most of the others were found no further than 15 m by the end of the experiment. When an insect was found on a plant, it was generally observed to stay there until the plant was completely defoliated, often with up to 15-20 beetles congregating on the same plant. Insects could walk 5-15 m in 24 h, but only around 50% of 103 insects after 48 h had found a host plant. This suggests that if *C. asclepiadeus* were to be released in NA, several release points would be necessary to effectively cover the target area.

In addition to beetle dispersal, our study shows that *C. asclepiadeus* will defoliate more leaf tissue area under partial and no sun than under full sun conditions. There are two main reasons why beetles might be consuming more plant material under shady conditions, namely, the effect of temperature and/or the effect of light. Temperature can have a serious effect on small poikilothermic animals like beetles. Some beetle species bury themselves in soil to maintain a lower body temperature, thereby conserving water and avoiding desiccation (Cloudsley-Thompson, 2001; Gunn, 1942). As *C. asclepiadeus* appeared to bury themselves under full sun conditions, it would seem they were spending fewer hours aboveground than beetles in shady conditions. Fewer hours aboveground would mean that *C. asclepiadeus* in full sun had fewer hours to feed, resulting in lower defoliation of leaf tissue area. Cooler and moister temperatures in shady conditions would allow *C. asclepiadeus* to remain aboveground for longer, continuing to eat leaf tissue. Sunny conditions decrease the number of hours available to feed, but not the rate at which *C. asclepiadeus* feeds. Beetle consumption of leaf tissue area was

also dependent on species: *V. nigrum* experienced less damage than *V. rossicum* or *V. hirundinaria*. It is likely that these differences are based on differences in size rather than *C. asclepiadeus* herbivory preference. The large size of *V. nigrum* compared to *V. rossicum* and *V. hirundinaria* suggests that the *V. nigrum* plants used in the experiment were older. As *Vincetoxicum* spp. are perennial, it is difficult to ensure that all plants collected are of the same age. If *C. asclepiadeus* were eating the same amount of leaf per capita on all three plants, but *V. nigrum* was larger, damage levels on *V. nigrum* would be expected to be lower. *Chrysochus asclepiadeus* were not eating less total mass of *V. nigrum*, but rather inflicted lower damage levels because defoliation was measured as a percentage of aboveground tissue lost.

In the third experiment, herbivore pressure on the plants also had an effect on the production of fruits and seeds, which can be described as an indirect effect because herbivory in this case was not directed against these specific organs. The treatments had an effect on fruit size of *V. rossicum* and on seed development (data not shown). Given the number of fruits produced per plant, the separate treatments on leaves and roots (L and R) had a negative impact, which is similar, and this impact was even greater when both treatments were combined. The effect of the two treatments appears to be synergistic. Treatments can then be arranged according to the importance of their impact on follicle development with herbivory on roots and leaves having the greatest effect, followed by treatment on roots only, and finally by leaves only. Herbivory had the same effect on seed production and development (data not shown).

Our results show an indirect effect of herbivory on the target plants, an effect not always seen in ecological studies of biocontrol agents and this has implications for successful implementation of biological control strategies. For example, if a seed feeder such as *Euphranta connexa* (Fab.) (Dipt.: Tephritidae) was to be used for control of swallow-worts, it should not be released into shady areas where the number of follicles is small or where the root and leaf herbivory is already sufficient to impact plant sexual reproduction. In shady areas it would seem more important to focus on phytophagous insects affecting leaves and roots than on those that will impact reproductive structures.

Classical biological control with herbivorous insects to control the expansion of an invasive plant requires many years of study, but once set up, can be more effective than repeated short-term measures (McFadyen, 1998). For targets such as swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*), high plant densities and large areas covered in regions of introduction present a major obstacle to success; however, such challenges could be met by releasing herbivorous insects in small numbers strategically at targeted locations. Thus, it is important to couple comparative studies on the ecology of the system and potential for biological control that include simulated herbivory in the invaded area (Milbrath, 2008; Doubleday and Cappuccino, 2011) as well as natural herbivory in the native area (Gassman et al., 2010; Sforza, 2009; Weed and Casagrande, 2010; Maguire et al., 2011) in order to make a final decision as to whether a biocontrol agent can be developed for use. Such studies should include measuring the potential for an insect to control the target plant, but also to limit its unintentional risks to non-target species. The stakes for success in systems such as swallow-worts are very high because these plants, currently a major problem in forested ecosystems, particularly in Canada and the northeastern USA, continue to invade new habitats outside of forests throughout North America, including grasslands, farmlands and urban areas.

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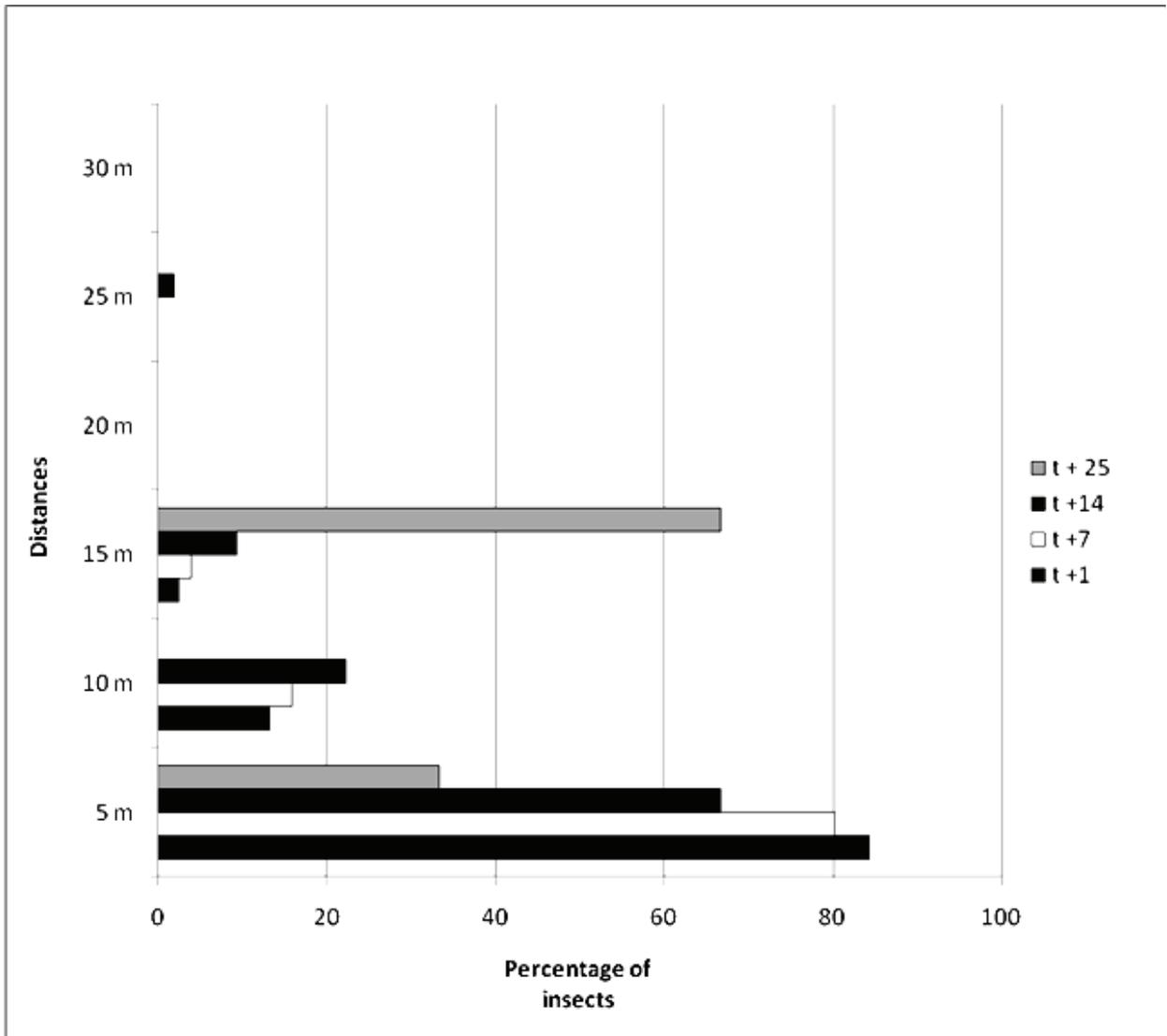


Figure 1. Dispersal activity of adults of *Chrysochus asclepiadeus* (Col.: Chrysomelidae) observed from 14 July to 9 August 2010, in percentage of alive insects observed for both sexes at 4 selected observations (t+1d, t+7d, t+14d and t+25d).

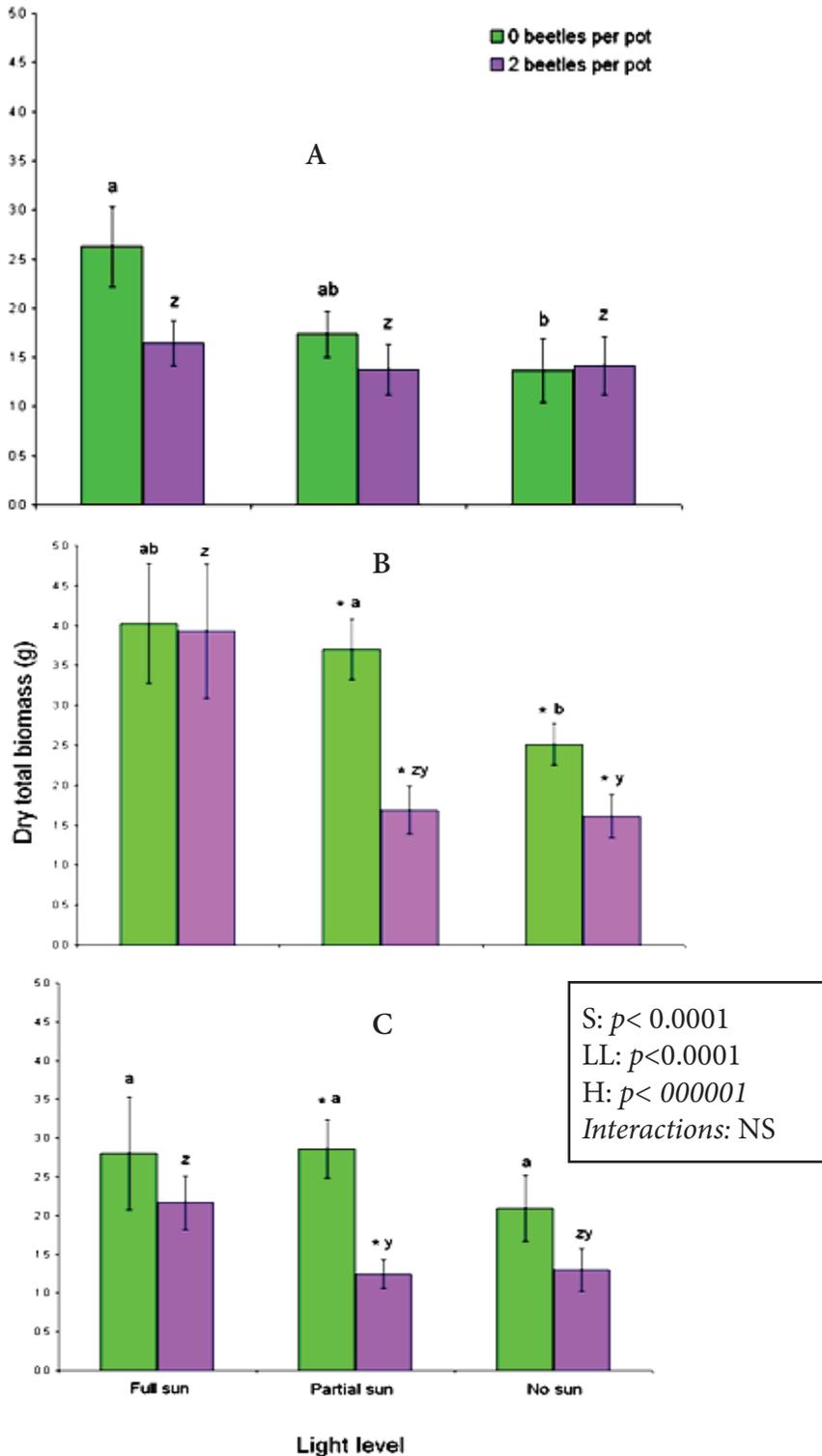


Figure 2. Total dry biomass (g) for A. *Vincetoxicum hirundinaria*, B. *V. nigrum*, and C. *V. rossicum*. Biomass plotted against beetle density and light level for the shading and herbivory experiment conducted outside of Montpellier, France in June and July 2010. Within light level, an asterisk denotes significant difference; between light levels within herbivory treatment, no shared letters denote significant differences (Wilcoxon rank sum test, $p < 0.05$). The legend above the graph reports the results of a Scheirer-Ray-Hare test in R (S = Species; LL = Light Level; H = Herbivory). Bars represent significant error.

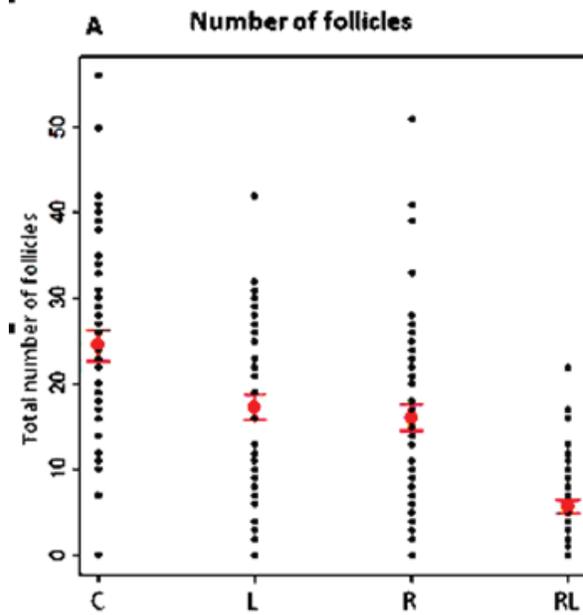


Figure 3. Number of follicles at harvest by treatment. Treatments: C = control; L= simulated herbivory on leaves; R = simulated herbivory on roots; RL = simulated herbivory on leaves and roots.