IMPACT OF THE DEFOLIATOR *CLEOPUS JAPONICUS* (COLEOPTERA: CURCULIONIDAE) ON *Buddleja davidii* IN THE LABORATORY

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ABSTRACT

Cleopus japonicus Wingelmüller (Coleoptera: Curculionidae) has been investigated as a potential biocontrol agent against *Buddleja davidii* Franchet (Buddlejaceae), an invasive weed of exotic and indigenous forests in New Zealand. The impact of feeding damage by *C. japonicus*, at densities of 0, 3, 10 or 20 larvae per plant (from 2nd instar), on the growth of buddleia plants was assessed in a quarantine laboratory. Matured larvae were replaced every 11 days until, after seven weeks, the effects of defoliation were assessed. Grazing damage increased with larval density and in the high density treatment (20 larvae per plant), 83% of expanded leaves had more than 50% of the leaf area grazed. One third of the plants in the high density treatment died, presumably as a result of grazing, whereas there was no mortality in the control and light grazing (3 larvae per plant) treatments. Grazing resulted in a significant reduction in main stem height, total stem and branch length, and dry weight of roots and shoots, primarily in the medium and high density treatments. The results suggest that, following permission to release and successful establishment, *C. japonicus* should successfully suppress buddleia growth.

Keywords: biological control, weeds, *Buddleja davidii*, *Cleopus japonicus*, Curculionidae, impact.

INTRODUCTION

Buddleja davidii Franchet (Buddlejaceae, buddleia) is a perennial woody shrub of central Chinese origin that is grown as an ornamental in many countries. In New Zealand, buddleia is now naturalised, occurs widely in natural and disturbed habitats in the North Island and the northern half of the South Island, and is recognised as a plant pest in several regions (Webb *et al.* 1988; Roy *et al.* 1998). Invasion of national parks and other protected areas by buddleia (e.g. Smale 1990) made it the subject of several control projects by the Department of Conservation (Kay and Smale 1990; Owen 1998). Along with several other invasive plants, buddleia is a major competitor for light and can cause growth losses and even increased mortality of seedlings in plantation forests (e.g. Richardson *et al.* 1996; 1999).

To reduce the negative effects of competition from weeds, herbicides are commonly applied during the establishment phase of plantation forests (Richardson 1993; Davenport 1995). Biological control (biocontrol) is a more sustainable method that can provide long-term control of specific targeted weeds. Using host-specific phytophagous insects or fungi, biocontrol is safe and has a good success rate, especially when exotic plants are the target (Julien and Griffiths 1998; McFadyen 1998). Buddleia appears to be a suitable target for a biocontrol programme (Kay and Smale 1990), and two weevils reared from buddleia in China have been identified as promising, host-specific agents. Colonies of these two species, the defoliator *Cleopus japonicus* Wingelmüller (Coleoptera: Curculionidae) and the stem borer *Mecyclobus erro* Pascoe (Coleoptera:

Curculionidae), are being investigated in quarantine at Forest Research, Rotorua. Both larvae and adults of *C. japonicus* graze the upper leaf surface, but most damage is done by the larvae. Pre-release studies of its phenology (Zhang *et al.* 1993) and host-specificity (M. Kay *et al.* unpubl.) have been completed, and an application to the Environmental Risk Management Authority for permission for its release is currently being prepared. An important aspect of the pre-release evaluation of a biocontrol agent is to determine its likely effectiveness in controlling the target weed. Here, we report the impact of various degrees of defoliation by *C. japonicus* larvae on several growth parameters of *B. davidii* seedlings in the laboratory.

MATERIALS AND METHODS

Insects

Larvae of *C. japonicus* used in this study were obtained from a laboratory colony established with beetles originating from *B. davidii* from Zhang Jia Jie National Forest, Hunan province, Peoples Republic of China, and reared on *B. davidii* plants in quarantine at Forest Research, Rotorua, since 1992. To obtain larvae for the study, multiple pairs of reproductively mature adults were placed together with cut *B. davidii* foliage in perspex containers with a mesh inset in the lid. Beetles were allowed to lay eggs on the foliage and were then removed. After a further 7 days, 2nd instar larvae were placed onto the study plants.

Host plants

Seedlings of *B. davidii* were grown from seed originating from a Rotorua population. Seeds were sown in October 1998 into tubes containing potting mix with slow-release fertiliser. At the two-leaf stage seedlings were thinned out. At 6 weeks old, plants were transplanted into 9 x 9 cm pots containing potting mix with slow release fertiliser, hardened off in a shade house for 2 weeks, and in the open for 1 week, before being transferred to the laboratory for experiments.

Impact of *C. japonicus* larval feeding

This experiment was conducted in a quarantine laboratory at $23 \pm 3^\circ\text{C}$ with Mercury vapour and incandescent lights providing 8 lux or $48 \text{ mmol m}^{-2} \text{ s}^{-1}$ PAR at a photoperiod of L:D 12:12. In December 1998, 2nd instar larvae of *C. japonicus* were transferred by camel-hair brush onto the upper surface of a randomly selected leaf of a *B. davidii* plant to create four different larval densities: (1) no larvae (control), (2) three larvae per plant, (3) 10 larvae per plant, or (4) 20 larvae per plant. Larvae began to pupate after 9 days of feeding, and after 11 days, all pupae and any remaining larvae were removed from each plant. They were replaced with a new cohort of second instar larvae. Each treatment was replicated six times, with plants of the same treatment arranged in groups of three in plastic trays. Plants were spaced at ca. 20 cm or more so they did not touch each other. Trays were rotated weekly to minimise position effects. Plants were hand-watered every other day.

Every 7 days, the height of the main stem from the soil to the growing tip was measured. After 7 weeks (i.e., five cohorts of larvae having fed on the plants), the experiment was terminated. For each *B. davidii* plant, the number of expanded leaves (i.e., longer than 3 cm without petiole) and the maximum length of the main stem and all branches were recorded. We also recorded the number of expanded leaves without feeding damage, and with < 50% and > 50% of the leaf area grazed for each plant. The soil around the roots of each plant was removed by washing, and all roots, stems, and leaves were dried separately in an oven at 50°C for 7-14 days to determine their dry weight to the nearest 10 mg.

Statistical analyses

The relationship between larval density and the proportion of leaves with heavy grazing was assessed using linear regression. To assess the effect of grazing on final plant height and dry weight, we included the plant height at the beginning of the experiment as a covariate in analyses of covariance (ANCOVA). This was necessary because there was some variation in plant height at the beginning of the experiment, and because both final plant height and final dry weight were significantly correlated with initial plant height (in control plants). ANCOVAs were followed by Tukey tests

to separate means. Residual plots had been examined to verify homogeneity of variance and normal distribution. SYSTAT 5.0 was used for all analyses.

RESULTS

The proportion of leaves with serious grazing damage from *C. japonicus* increased significantly with larval density (regression; $df = 1, 22$; $MS = 13981.7$; $F = 42.2$; $P < 0.001$; $r^2 = 0.66$) (Fig. 1). In the treatment with high larval density (20 larvae per plant), $83\% \pm 7\%$ (mean $\pm$ SE) of leaves suffered over 50% loss of the photosynthetic area. A few small holes in some leaves of four of the six control plants (Fig. 1) had been caused by a temporary attack by a few adult beetles (not larvae).

FIGURE 1: Mean number ($\pm$ SE) of *B. davidii* leaves with none, or less or more than 50% of the leaf area grazed and the total number of leaves on plants exposed over 7 weeks to grazing by *C. japonicus* at 0, 3, 10 or 20 larvae per plant.

After five weeks of grazing (two weeks before the experiment was terminated), two plants in the 'high density' treatment, as well as one plant in the 'medium density' treatment died, presumably as a result of the sustained defoliation, whereas there was no mortality in the control and 'low density' treatments. Grazing resulted in a moderate, statistically significant reduction in main stem height (ANCOVA; grazing: $df = 3$, $MS = 462.0$, $P = 0.001$; initial height (covariate): $df = 1$, $MS = 2687.9$, $P < 0.001$; error: $df = 19$, $MS = 53.6$) (Fig. 2). There was a more marked effect when branches were included to assess the mean total stem length, which was 124.2, 101.8, 92.3 and 64.3 cm for the control, low, medium and high density treatments, respectively (ANCOVA; grazing: $df = 3$, $MS = 3562$, $P = 0.033$; initial height (covariate): $df = 1$, $MS = 9280$, $P = 0.006$; error: $df = 19$, $MS = 988$). These results are conservative because about 10 cm broke off the main stem of one control plant due to handling three weeks into the experiment.

Plant dry weight at the end of the experiment decreased significantly with an increase in larval density (ANCOVA; grazing: $df = 3$, $MS = 10.2$, $P = 0.001$; initial height (covariate): $df = 1$, $MS = 36.2$, $P < 0.001$; error: $df = 19$, $MS = 1.3$) (Fig. 3). The dry weight of plants in the 'high density' treatment was only half that of control plants. This was caused by a concomitant effect on above-ground and root biomass, i.e. no difference in the root:shoot ratio.

FIGURE 2: Mean height ($\pm$ SE) of main stem of *B. davidii* plants exposed over 7 weeks to grazing by *C. japonicus* at 0, 3, 10 or 20 larvae per plant. Means not sharing a letter are significantly different at $P < 0.005$ (ANCOVA, Tukey test, see text).

FIGURE 3: Mean dry weight ($\pm$ SE) of *B. davidii* plants exposed over 7 weeks to grazing by *C. japonicus* at 0, 3, 10 or 20 larvae per plant. Means not sharing a letter are significantly different at $P < 0.042$ (ANCOVA, Tukey test, see text).

DISCUSSION AND CONCLUSIONS

This study showed that feeding damage by *C. japonicus* can result in a significant reduction in stem length and biomass as well as death of buddleia plants. To assess whether this allows predictions about the potential effectiveness of *C. japonicus* as a biocontrol agent against buddleia, several issues need to be considered, ranging from likelihood of agent establishment to the definition of success. Assuming that permission to release will be granted, *C. japonicus* would probably establish and acclimatise well in the areas where buddleia is abundant, even in cooler regions such as the central North Island (Zhang *et al.* 1993). Under these climates, *C. japonicus* will probably have 2-3 generations per year (Zhang *et al.* 1993). Also, host plants are abundant and

there are no specialised natural enemies of *C. japonicus* in New Zealand. In a natural habitat, *C. japonicus* populations would thus have the opportunity to build up rapidly (whereas the number of larvae per plant was kept constant in this experiment). It is reasonable to expect that populations of *C. japonicus* could reach the larval densities required for a substantial growth reduction and death of plants. However, the exact population size of *C. japonicus* required is difficult to predict from our laboratory study because control plants reached only about one quarter of the dry weight of similar plants growing in the field, although differences in height growth were small (unpubl. data). This was probably due to less favourable light in the laboratory and limitations on root growth in pots.

In plantation forests, harvesting and site preparation cause major disturbances of the habitat, which has important implications for biocontrol. Buddleia quickly invades these cutover sites with its wind dispersed seeds, and grows rapidly in height at over 1 m per year during the first three years (Richardson *et al.* 1996). It is during this time that competition from buddleia has the most serious effects on the growth of *Pinus radiata* D. Don., and *C. japonicus* would have to catch up quickly with the buddleia invasion after habitat disturbance. However, to be successful, it is not necessary to suppress buddleia entirely. Rather, only a certain reduction in buddleia growth would be required to decrease the competition with *P. radiata* to a negligible level. For example, if buddleia height is less than 60% of tree height, the presence of this weed is not directly detrimental (B. Richardson, pers. comm.), but this value might change if a substantial reduction in leaf area of buddleia could be achieved. A growth and defoliation modeling approach could be used to determine the number of *C. japonicus* females required per m² infested area to achieve sufficient weed control (Zabkiewicz *et al.* 1998).

Additional suppression of buddleia could be achieved because sustained grazing damage would probably also result in a reduced seed production. This was demonstrated for defoliating beetles on a perennial shrub in northern Australia (Lonsdale *et al.* 1995). The control in New Zealand of *Hypericum perforatum* L. with the St. John's wort beetle, *Chrysolina hyperici* (Forster) (Coleoptera: Chrysomelidae) demonstrates that a single defoliator agent can be successful (Syrett and Hancox 1985). However, most weed biocontrol projects require more than one agent. Thus, the prospects for successful biocontrol of buddleia will increase with the planned release of a second biocontrol agent, the stem borer *Mecynotus erro* which is currently under investigation.

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