TWO-PHASE OPEN-FIELD TEST TO CONFIRM HOST RANGE OF A BIOCONTROL AGENT *CLEOPUS JAPONICUS*

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ABSTRACT

The buddleia leaf weevil, *Cleopus japonicus*, was released in New Zealand in 2006 as a biological control agent for the weed *Buddleja davidii*. A two-phase open-field design was used to confirm laboratory host range and examine non-target impacts in the field. This was the first field trial undertaken in New Zealand and included six non-target plant species. Feeding and dispersal of the agent on the test species and *B. davidii* were compared. *Cleopus japonicus* strongly preferred *B. davidii*. Larvae were recorded on *Verbascum virgatum* and *Scrophularia auriculata* during the choice stage of the trial. Killing the *B. davidii* plants in the second phase resulted in adults feeding on the two exotic species, *V. virgatum* and *S. auriculata*. Minor exploratory feeding was recorded on the natives *Hebe speciosa* and *Myoporum laetum*. These results confirm that laboratory tests conducted to assess the safety of this agent for release in New Zealand accurately predicted field host range. **Keywords:** buddleia leaf weevil, *Cleopus japonicus*, *Buddleja davidii*, feeding, non-target effects, field trial.

INTRODUCTION

In a paper by Barratt et al. (2009) reviewing risk assessments of classical biological control in New Zealand, one of the case studies used was that of the agent *Cleopus japonicus* Wingelmüller (Coleoptera: Curculionidae) against *Buddleja davidii* Franchet. The Environmental Risk Management Authority (ERMA) raised questions over the safety of this agent because during the original host specificity testing one *C. japonicus* larva was reared to adult on a culturally important (taonga) species of native plant *Hebe speciosa* (A. Cunn.) Cockayne & Allan. No further individuals developed on *H. speciosa* or any other *Hebe* species tested (Kay & Hill 2005). This field study was conducted to investigate whether the pre-release laboratory host tests reliably predicted subsequent host range, and to discover whether there was indeed any real risks to *H. speciosa*.

Consistent with current thinking, hosts of *C. japonicus* in New Zealand are expected to be those most closely related to the target weed *B. davidii* (Sheppard et al. 2005). However, the taxonomy of *Buddleja* is unclear, having been placed in the Scrophulariaceae, Loganiaceae, Buddlejaceae and most recently into the Scrophulariaceae once again (Tank et al. 2006). The laboratory testing predicted that apart from ornamental *Buddleja* (Scrophulariaceae) species, plants most at risk from non-target attack by *C. japonicus* in New Zealand would be: (a) two exotic weed species within the Scrophulariaceae *sensu lato*, *Scrophularia auriculata* L and *Verbascum virgatum* Stokes, followed to a much lesser extent by (b) *Myoporum laetum* G. Forst. (Myoporaceae) – the only indigenous New Zealand species in the same clade as *B. davidii* (Tank et al. 2006; Kay et al. 2008), (c) species within the *Hebe* genus, which has recently been placed within the Scrophulariaceae *sensu lato* clade and (d) the native species falling within the newly established clade Veronica (*Limosella lineata* Glück (Limosellae) and *Glossostigma*

elatinoides Benth. ex Hook.f (Phrymaceae)). It was predicted that *C. japonicus* larvae, rather than adults, posed the most immediate non-target impact threat. Larvae have a limited potential to spill over, either by dropping from the canopy to foliage below or by moving to plants located in the immediate vicinity. This is likely to only occur when nearly complete defoliation of their *B. davidii* host plant has occurred. It is this scenario that appears to have the highest risk of non-target impacts from *C. japonicus*, and puts plants of vastly different form from *B. davidii*, such as semi-aquatic, stream-side and ground-cover plants at risk from spill-over. In 2006/2007 the predicted risk was examined by undertaking a two-phase open-field host-specificity trial (Briese 1999) to confirm laboratory host range and examine non-target impacts in the field.

MATERIALS AND METHODS

Plant sources

Seven plant species, including *B. davidii* were chosen for inclusion in the trial. Two species, *B. davidii* (target) and moth mullein weed *V. virgatum*, were known from laboratory tests to be full hosts for *C. japonicus*. The remaining plants were found to be partial hosts. These were the weed, *S. auriculata* (which supported adult feeding and oviposition in laboratory tests); the native aquatic species *G. elatinoides* (supported larval feeding) and *L. lineata* (supported adult feeding and moderate larval feeding); and the natives *H. speciosa* (minor exploratory feeding, but one larva pupated) and *M. laetum* (minor oviposition recorded) (Kay & Hill 2005).

Hebe speciosa was grown from cuttings from the Landcare Research collection growing at Lincoln, Canterbury. These plants were originally collected from seed in 1977 from either Mokau in Taranaki or Titirangi Bay in the Marlborough Sounds (Phil Garnock-Jones via David Given). *Myoporum laetum* plants were nursery-grown from seed collected from the Waitakere Ranges, Auckland. *Buddleja davidii* was grown from seed collected from within the Scion grounds, Rotorua. All other non-target plants were transplanted from the field – *S. auriculata* from Kinleith Forest, Tokoroa, and *V. virgatum*, *L. lineata* and *G. elatinoides* from Lake Okataina, Bay of Plenty.

Trial design

A single plot was planted into a field that had been rotary hoed in November and left to fallow until planting in January. The plot had eight rows containing one of each of the seven plant species planted 1 m apart (56 plants in total). The position of each plant species in each row was designed so that two plants of the same species were never adjacent. An irrigation system was established to provide watering as needed. Additional soil moisture requirements for aquatic species were catered for by planting *S. auriculata* within submerged 10 litre plastic buckets and planting *G. elatinoides* and *L. lineata* in shallow ponds created from plastic so that the foliage was out of the water. No plant canopies were overlapping. A phase set-design experiment from choice to no-choice was chosen (Briese 1999; Briese et al. 2002). The choice phase involved releasing *C. japonicus* within the trial in January 2007 and quantifying *C. japonicus* presence on, and damage to, the target and non-target plants. The no-choice phase involved killing the *B. davidii* plants at day 77 and quantifying *C. japonicus* movement onto, and any subsequent damage to, non-target plants.

A. Choice phase: pre-ovipositing adult establishment and dispersal

In this choice phase, ten *C. japonicus* pupae were placed at the base of each of the 56 plants in the field plot in January and subsequently both the number of adults and larvae, as well as plant damage, were recorded weekly (where 0=no damage, 1=<2%, 2=2-10%, 3=10-50%, 4=50-90% and 5=>90% of plant affected).

B. No-choice phase: dispersal following host plant death

The number of adult *C. japonicus* on each of the eight source *B. davidii* in the plot was counted and additional adults added to make a total of 40 adults on each *B. davidii* plant. This was done to ensure evenly spread population dispersal at the start of the second phase. To monitor dispersal in the no-choice phase, 26 *B. davidii* were planted on day 77 around the field plot perimeter, 3 m away, to act as trap plants. On the same

day, each of the eight source *B. davidii* within the field plot were ring-barked in an attempt to kill them, but the plants only wilted, so on day 86 all *B. davidii* stems were cut off at ground level. Severed stems were pushed into the soil alongside the stump so the dead and desiccated plant remained upright to enable the *C. japonicus* to disperse in a natural manner. The number of larvae and adults present on the desiccated source *B. davidii*, non-target plants and the surrounding trap plants, was recorded weekly until the trial was concluded on day 120.

Data analysis

Data on larval and adult abundance were analysed for effect of plant species using a Generalised Linear Model in SAS and where significant differences existed, means were separated by a Student Newman-Keuls Test.

RESULTS

A. Choice phase: pre-ovipositing adult establishment and dispersal

The number of both adults and larvae on *B. davidii* within the field plot was significantly greater than on the non-target species ($P < 0.001$ and $P < 0.0001$, respectively) on all sampling dates (Fig. 1). A maximum of 12.6 *C. japonicus* adults per *B. davidii* plant was recorded on day 11, the number then declined over time. Seven days after the trial began, there was a mean of 0.86 adult *C. japonicus* on *V. virgatum* and 0.5 adults per plant on *S. auriculata*, with a second peak on this species of 1.0 adults per plant on day 42, after which numbers declined (Fig. 1). Single adults were recorded on individual *H. speciosa* and *M. laetum* plants early in phase A following emergence, with no adults recorded on these species after day 17.

FIGURE 1: Mean ($\pm$ SE) number of *C. japonicus* adults per plant recorded on each plant species following the release of pupae at the base of all test plants on day 0.

Cleopus japonicus larvae were first recorded on the *B. davidii* plants on day 49 and numbers peaked on day 77 at 54.6 larvae per plant (Fig. 2). Twelve larvae were recorded on one *V. virgatum* plant on day 49, and three larvae on a second *V. virgatum* plant on day 63 equating to a mean never exceeding 3.0 larvae per plant. A single pupa was recorded on *V. virgatum*.

FIGURE 2: Mean ($\pm$ SE) number of *C. japonicus* larvae per plant recorded on each plant species following the release of pupae at the base of all test plants on day 0.

B. No-choice phase: dispersal following host plant death

Adults and larvae remained on the killed *B. davidii* source plants for 2 weeks after the plants were killed. Larvae eventually either pupated or died from starvation. In the days after the source *B. davidii* were killed, the greatest number of adult *C. japonicus* were recorded on *S. auriculata*, with a maximum of 1.8 adults per plant recorded on days 103 and 110 (Fig. 3). A mean of 0.13 adults per *V. virgatum* were recorded weekly following the death of *B. davidii*. Notably this arose from single adults recorded on different individual *V. virgatum* plants. A single adult was also detected on *M. laetum* on day 92.

FIGURE 3: Mean ($\pm$ SE) number of *C. japonicus* adults per plant recorded on each plant species. Measurements were made after additional adults were added to *B. davidii* to make 40 per plant, and the *B. davidii* stems ring-barked, on day 77. On day 86 the stems of *B. davidii* were severed.

A single larva was detected on *S. auriculata* on day 110 but was not present after day 124. It is unknown whether this larva pupated. No larvae were ever detected on *H. speciosa* or *M. laetum* at any stage of the experiment. Larvae were first detected on the outer trap *B. davidii* plants on day 109, 32 days after these plants were planted, by which time an average of 6.2 larvae per plant was recorded (Fig. 2). The number of larvae on these plants continued to increase until monitoring ended on 28 May 2007.

Moderate feeding damage (2–10%) was recorded on *S. auriculata* for the two stages of the field trial and in phase A for *V. virgatum* (Table 1). Whilst exploratory feeding damage was recorded on the two natives *H. speciosa* and *M. laetum* in phase A, no feeding was observed on these species in phase B, or on *L. lineata* or *G. elatinoides* throughout the trial (Table 1).

TABLE 1: Average feeding damage to each plant species before and after *B. davidii* plants were killed, where 0=no feeding damage, 1=<2%, 2=2–10%, 3=10–50%, and stages of *Cleopus japonicus* development recorded on each species.

Host species	Feeding damage before <i>B. davidii</i> source plants removed	Feeding damage after <i>B. davidii</i> source plants removed	Development stage responsible for damage
<i>Buddleja davidii</i> source plants	3	--	adults, larvae
<i>Verbascum virgatum</i>	2	1	adults, larvae
<i>Scrophularia auriculata</i>	2	2	adults, 1 larva
<i>Hebe speciosa</i>	1	0	adult
<i>Myoporum laetum</i>	1	0	adult
<i>Glossostigma elatinoides</i>	0	0	none
<i>Limosella lineata</i>	0	0	none

DISCUSSION

The application to ERMA to release *C. japonicus* into the field in New Zealand concluded that even though *H. speciosa* supported complete development of the weevil in one instance, the risk that the control agent would produce self-sustaining populations on environmentally and economically valued plants was low (Kay et al. 2008). ERMA eventually accepted this conclusion and authorised release. This two-phase open-field post-release study confirms that the field host-range predicted by the original laboratory studies (Kay et al. 2008) was sound. It is known that the host selection behaviour of an agent can differ when the target weed is present and absent (Blossey et al. 1994; Fowler et al. 2003), yet *C. japonicus* significantly colonised *B. davidii* compared with other non-target hosts present in both phases of the field trial.

Laboratory testing predicted minor feeding damage on the weeds *S. auriculata* and *V. virgatum* (Kay et al. 2008), and damage was seen in this field trial to not exceed 10% of leaf area (Table 1). In laboratory no-choice tests, feeding damage on *V. virgatum* was ranked equally to *B. davidii*, but only one out of five larvae survived to pupation (Kay & Hill 2005). In the present field trial only one *C. japonicus* was recorded to have pupated on *V. virgatum*. Feeding damage on *S. auriculata* was moderate in laboratory no-choice tests, compared to *B. davidii*, and larval survival was not tested (Kay et al. 2008). In the present field trial only a small number of adult *C. japonicus* and one larva were recorded on *S. auriculata*. The data presented confirm that the methods used to predict host range of this weed biological control agent were robust, and these predictions were realistic.

Discriminating between *C. japonicus* feeding damage and other herbivore damage was difficult in some cases, as adult damage is similar to damage caused by other insects, such

as some Lepidoptera and other Curculionidae. Clement & Cristofaro (1995) identified this as a potential logistical restraint to the implementation of open-field host-specificity testing, and we had the same experience. For instance, the causal agent for the leaf damage recorded on *M. laetum* and *H. speciosa* during the field trial could not be ascertained. Because adult *C. japonicus* were recorded on both *M. laetum* and *H. speciosa* it must be reported that *C. japonicus* was responsible. If indeed *C. japonicus* did damage *M. laetum* and *H. speciosa* then the holes equated to exploratory feeding only.

In phase A, the *C. japonicus* were released as pupae, to allow for the natural pre-alighting cues of adult weevils in choosing between *B. davidii* and the test plants following emergence. Furthermore, newly emerged adults can be expected to show the highest motivation to feed because this is the life stage when nutritional requirements for food are greatest (Gresham et al. 2009). The presence of a small number of *C. japonicus* on *S. auriculata*, *V. virgatum*, *H. speciosa* and *M. laetum* early in phase A of the trial may be a product of both this high feeding motivation, and agent mobility. Weevils are typically poor dispersers (Briese 1999) and data have not yet been collected on the flight ability of *C. japonicus*.

In phase B, the source *B. davidii* plants were killed to simulate the death of the target host. *Cleopus japonicus* has been predicted to completely defoliate *B. davidii* at some sites (Kriticos et al. 2009). The observation in the present trial that many adults remained for up to 24 days on the dead and desiccated *B. davidii* plants rather than dispersing confirmed *B. davidii* as the preferred host. *Scrophularia auriculata* received the most non-target attack following death of the *B. davidii*. This was caused by adult *C. japonicus* dispersing from the dead *B. davidii* before they were able to locate host plants further away.

The results of this two-phase open field trial suggest that the proximity of the non-target plants to infested *B. davidii* will be important in determining the likelihood of non-target impacts. *Cleopus japonicus* larvae have limited dispersal and will only survive after death of the host plant if they can pupate prematurely or are able to move directly onto another host plant. No larvae were found on non-target plants immediately after the death of *B. davidii* suggesting the limit to larval spill-over in the field is less than the 1 m separating plants in the field trial. Therefore, it is predicted that damage to non-target plants by *C. japonicus* larvae will only occur when the canopy of a dying or defoliated *B. davidii* is over-hanging a palatable non-target plant.

In conclusion, the results of this field trial confirm that laboratory tests conducted to assess the safety of *C. japonicus* for release in New Zealand accurately predicted its field host range, and that no native plants are under any significant threat of non-target attack.

ACKNOWLEDGEMENTS

This work was funded by New Zealand's Foundation for Research, Science and Technology through the Better Border Biosecurity (B3) Programme. Many thanks to Sam Brown and Dylan Cox for technical assistance and Mark Kimberley for statistical analyses.

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