Strategies for Achieving Widespread Establishment of Broom Seed Beetle, Bruchidius villosus (Coleoptera: Chrysomelidae), a Biological Control Agent for Broom, Cytisus scoparius, in New Zealand

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Abstract

To achieve maximum efficacy of weed biological control introductions, it is important that the full benefits are realized as early as possible. The aim of experiments described here was to show how to gain widespread establishment of broom seed beetle (Bruchidius villosus (F.)) as quickly as possible. Broom seed beetle was introduced into New Zealand as a biological control agent for broom (Cytisus scoparius (L.) Link) in 1988, and since 1991 beetles have been released at 98 sites throughout the country. By summer 1998-99 the beetles had established in at least 23 of these sites. Key factors for establishing beetle populations in the field are the successful overwintering of adult beetles, and the synchrony of beetle development with flowering and pod development of broom. Methods are described for mass rearing beetles that require low labour input and in which 70% of overwintering beetles survive. Reproductively active beetles have usually been released in spring or early summer when appropriate stages of broom pods are available for oviposition, but new generation beetles have also been recovered from populations released in late summer, when beetles cannot reproduce until the following season. Beetles collected from the field are more abundant and easy to find for transfer to new sites in late summer or autumn, than in spring. Of several collection methods tested, the most efficient was to collect infested seed in autumn. This straightforward redistribution method can be used by farmers, foresters, and conservation managers.

Keywords: broom, broom seed beetle, Cytisus scoparius, Bruchidius villosus, biological control, agent establishment, New Zealand

Several insects introduced into New Zealand for weed biological control are still limited in distribution, or have taken many years to spread to weed infestations throughout the country (e.g., Botanophylla jacobaeae (Syrett 1989), Chrysolina quadrigemina (Fraser and Emberson1987), and Leucoptera spartifoliella (Syrett and Harman 1995)). Since the development of technology transfer programs in New Zealand, releases of weed biological control agents have been more numerous and more widely distributed, and establishment rates have increased (Hayes 1999b). Research has been conducted for users of these programs, in association with research funded by central government, to improve the uptake and effectiveness of weed biological control. In this paper we describe work that is enhancing the rapid and widespread establishment of a seed-feeding beetle for sup-
pression of the shrub weed broom, *Cytisus scoparius* (L.) Link (Fabaceae).

A biological control program for *C. scoparius* began in New Zealand in 1981 (Syrett 1996). Broom seed beetle (*Bruchidius villosus* (F.) (Coleoptera: Chrysomelidae) develops in the seeds of *C. scoparius*. It is a European species that is also established in the eastern USA (Bottimer 1968). Both *B. villosus*, and another European seed-feeding species, *Exapion fuscirostre* (F.) (Coleoptera: Curculionidae), were considered for introduction into New Zealand (O’Donnell and Manfield 1986). The population biologies of the two beetles were investigated by Parnell (1966), and an attempt to study their interactions was made by Hinz (1992). *Bruchidius villosus* was selected first as a potential biological control agent for *C. scoparius* in New Zealand because, unlike *E. fuscirostre*, its diapause is facultative, and beetles can be continuously reared under long days by providing broom pollen and suitable green pods for oviposition (H.M.H. personal observation). We predicted that this would enable *B. villosus* to be more easily synchronized with the reproductive development of *C. scoparius* (Harman 1999). *Exapion fuscirostre* was intentionally released, and is now well established in the western USA, as a biological control agent for *C. scoparius*. (Syrett et al. 1999).

Eggs of *B. villosus* are deposited by female beetles on the outside of green pods at least 32 mm long (Hinz 1992). The hatching larva bores through the pod into a developing seed (usually a single larva into a single seed) where it completes its own development through to the pupal stage, destroying the seed in the process. Adult beetles, emerging from the pupal stage, exit from the seed casing, often before the pod dehisces, and so beetles are explosively dispersed instead of seeds (H.M.H. personal observation). In the UK *B. villosus* is parasitized by several hymenopterous species (Parnell 1964), one of which, *Pteromalis sequester* Walker (Hymenoptera: Pteromalidae) is established in New Zealand (Valentine and Walker 1991), and attacks gorse seed weevil (*Exapion ulicis* Förster).

Tests to determine the host range of *B. villosus* were completed in the UK and in the insect containment facility at Lincoln, New Zealand (Syrett and O’Donnell 1987). The first shipment of adult beetles was sent from CABI Bioscience, Ascot, UK to Lincoln, in September 1985. The beetles were collected from *C. scoparius* growing at Silwood Park, Ascot, and were imported into the insect containment facility at Landcare Research (formerly the Department of Scientific and Industrial Research). Beetles from this shipment were used to complete host specificity tests on plants that did not produce pods in the UK (Syrett and O’Donnell 1987). These indicated that the beetle was restricted to *Cytisus* spp., and permission to release beetles was received from the Ministry of Agriculture and Fisheries in November 1987. A first, small, field release was made on 30 November on the Port Hills near Christchurch. Further shipments were received into containment from 1988 to 1991 (Wilcox et al. 1991), and the first substantial field releases were made in 1988 (Harman et al. 1996). Contrary to predictions from host test results, in 1998 beetles were recovered from field-collected seeds of *Chamaecytisus palmensis* (Christ) Bisby et K. Nicholls (Fabaceae) at Lincoln, New Zealand (Fowler et al. 1999). Beetles collected from New Zealand were released in Australia in 1993 (Syrett et al. 1999).

In this paper we describe the development of methods to rear *B. villosus* beetles, from rearing small numbers in the laboratory for field release, to a mass-rearing technique on field-grown plants, and a field collection method. Methods are also described for maintaining large numbers of beetles through the winter, for releasing beetles, and for collecting beetles from established field sites for redistribution to new sites. Data on release numbers, recovery, and establishment of beetles are also presented.
1: Rearing Beetles in Containment

Methods

It has proved difficult to rear beetles under artificial conditions because *C. scoparius* plants do not readily produce viable pods under artificial light, and without natural pollination. Hand pollination had very low success, and the use of bumble bee queens gave only slightly better results (H.M.H. unpublished data). Newly imported beetles were maintained in clear plastic containers (Freshpac®) (220 × 135 × 75 mm) lined with moistened filter paper and containing a sprig of flowering *C. scoparius* that also had at least one green pod of a size suitable for oviposition. The ends of the bouquet were wrapped in cotton wool secured by a piece of Parafilm®. Beetles were also provided with a commercial pollen source, which had been preserved frozen, and cotton dental rolls soaked in a honey-water solution as food supplements. They were held under long days (16 : 8 h photophase), at temperatures of 20 : 12°C, and 55 : 75 % RH. When female beetles began laying eggs inside the containers, they were transferred to potted plants bearing fully formed green broom pods suitable for oviposition (> 32 mm long). The plants were 1.5 m tall. Three plants were used, and 20 beetles were caged to a single branch bearing pods by means of a Terylene gauze sleeve (for details of sleeve construction, see below). Beetles were transferred daily to a new branch. Seeds were harvested from the plants when pods had matured, and new generation adults allowed to emerge naturally from the seeds. Some adult beetles failed to emerge naturally, but moistening the seeds facilitated beetle emergence.

Results

Female beetles commenced laying eggs 3-8 weeks after being supplied with broom flowers and green pods. We reared 117 beetles from an initial population of 108.

2: Rearing Beetles from Sleeved Cages on Field-Grown Plants

Methods

Between 1991-92 and 1994-95, rearing was conducted out of doors on field-grown *C. scoparius* bushes, 6-10 years old. Reproductively mature beetles were caged onto flowering *C. scoparius* branches in Terylene gauze cages. Branches were selected that had large numbers of flowers and suitable-sized pods. Gauze sleeves 600 × 300 mm in size, open at both ends, were fastened over the branches. One end of the sleeve was secured around the branch with a plastic twist-tie, the other was closed to include the end of the branch, and was also secured with a twist-tie. A plastic or paper plant label was attached to the outer end. Twenty beetles (10 in 1990-91) were placed inside each gauze cage, and left on the branch for 1 week. Sample groups (approximately 1 in 20) were checked to ensure the sex ratio was between 40 : 60 and 60 : 40 male : female. After 1 week beetles were collected from the sleeves, and placed in new sleeves on new branches. The used branches were marked with prominent paper plant tags for later identification, and labeled with the date and sleeve number. When the pods had ripened, but before they dehisced, the sleeves were replaced. In early January, once all pods had ripened, and had begun to dehisce, the sleeved branches were cut from the bushes and returned to the laboratory for recovery of the mature seed and new-generation beetles. Seeds with unemerged beetles inside were placed on a thin layer on absorbent paper towelling, on rectangular white plastic trays, 350×280 mm. Each tray was placed inside a Terylene gauze sleeve to retain emerging beetles while allowing adequate ventilation. Once the first beetles were observed emerging,
trays were watered 2-3 times per week. A flush of emerging beetles occurred about 30 min after wetting. Beetles were recovered from the trays every 2 d and transferred to clear plastic Freshpac® containers lined with moistened filter paper, and supplied with pollen and a honey-water solution as a food source. Some beetles were attacked by the parasitoid Pteromalis sequester. When numbers of beetles emerging from sleeves were recorded, parasitoid numbers were also noted.

Results
The numbers of beetles reared in successive years is presented in Table 1. More beetles were produced in 1993-94 than in earlier years because the sleeved rearing was supplemented by field-collected beetles. Less than 1% of beetles were affected by parasitoids. However, the wasps appeared to emerge from seeds earlier than the beetles and it is likely that some of them escaped from the gauze cages, so the infestation levels recorded may be an underestimate.

3: Field Collection to Obtain Beetles
Methods
In 1993-94 when high populations of beetles had established in the field, a low-input collection method was used to retrieve beetles in late summer for overwintering in the laboratory, and release at new sites the following spring. Branches of C. scoparius bearing large numbers of pods infested with beetles were cut from the field site, and removed to the laboratory. Here the pods and seeds were stripped from the branches, and placed in large Perspex boxes 0.75 × 0.5 × 0.5 m. Pods and seeds were misted regularly to facilitate emergence. An even lower-input method was adopted later: branches bearing pods were placed on benches in a small, insect-rearing room, where beetles emerged naturally, were attracted to the lights in the room, and could then easily be collected by aspirator from the glass screens in front of the lights, and the surrounding white ceiling.

Results
At least 40,000 additional beetles were collected using this method (Table 1).

<table>
<thead>
<tr>
<th>Year beetles reared</th>
<th>No. of sleeves</th>
<th>No. of beetles produced</th>
<th>% survival to following year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991-92 (experimental)</td>
<td>17</td>
<td>382</td>
<td>70</td>
</tr>
<tr>
<td>1991-92</td>
<td>400</td>
<td>14,000</td>
<td>52</td>
</tr>
<tr>
<td>1992-93</td>
<td>335</td>
<td>10,000</td>
<td>66</td>
</tr>
<tr>
<td>1993-94 + from field</td>
<td>600</td>
<td>53,000</td>
<td>70 (30)*</td>
</tr>
</tbody>
</table>

* Only 30% of beetles held for 26 wk under “winter” conditions (for release at later sites) emerged, compared to 70% for beetles held for 22 wk.
4: Maintaining Beetles through the Winter

Methods

Several regimes were used, depending on the number of beetles to be held through the winter. For smaller numbers of beetles, Freshpac® containers lined with filter paper were used. Larger numbers of beetles were maintained in specially constructed Perspex containers dubbed “apartment blocks” (Fig. 1). These containers were constructed to provide the maximum amount of surface area of material suitable for beetles to retreat into, and therefore to house a large number of beetles without creating conditions conducive to disease development. Each cage was 400 × 400 × 400 mm, and housed 3,000-6,000 beetles. Beetles were provided with pollen and dental rolls soaked in a honey-water solution for food. These were placed on a sheet of filter paper, and changed weekly.

Results

Survival was high (up to 70%, Table 1) using both types of container. However, there was a marked reduction in survival of beetles that were held under “winter” conditions for more than 22 wk. In order to synchronize the release of beetles with availability of suitable pods, it was necessary that for some sites, where development of *C. scoparius* was delayed, that beetles be retained for 4 wk longer than for the earliest sites. Survival of these beetles was much lower (30%).

5: Field Releases of Beetles

Methods

Two field releases, each of 500 adult beetles, were made in October 1988, on the Port Hills, Christchurch, and at Calf Stream, Glynn Wye Station, north of Hanmer Springs, North Canterbury. Subsequent releases are listed in Table 2, and locations are shown in Fig. 2. Ten releases each comprising 1,000 newly emerged beetles were made in late summer (in March 1996), as opposed to releasing overwintered beetles in spring. The release sites were all in the Amuri Basin, North Canterbury (Fig. 2). To determine whether smaller numbers of beetles would establish, releases of 500 and 1,000 beetles were made in October 1996 at 2 adjacent sites in each of 5 different places in New Zealand (Wanganui, Kinleith, Whakatane, Marlborough, and Clutha) (Fig. 2).

Fig. 1. Perspex container used for maintaining *Bruchidius villosus* beetles under laboratory conditions during winter (“apartment block”)
Table 2.
Releases of *Bruchidius villosus* beetles made between 1991 and 1998. Beetles are classed as established if increasing numbers of beetles are recovered in 2 successive years.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>23</td>
<td>26</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Established</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recovered</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Not known</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>9</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Failed</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Distribution of sites in New Zealand where *Bruchidius villosus* beetles have been released between 1988 and 1998.
Results

Beetles are accepted as having established at a site if they are recovered in increasing numbers in 2 successive years. Beetles failed to establish from the release made on the Port Hills, Christchurch, but a population has survived from the release made at Calf Stream, North Canterbury. The current status of releases made to 1998 is presented in Table 2. Beetles have been recovered from 8 of the 10 releases of 1,000 beetles made in March 1996, but establishment is not yet confirmed. Where 500 and 1,000 individuals were released, beetles were recovered from both sites in each of 4 of the 5 regions, and from neither of the 2 sites in the 5th region.

6: Redistribution methods

Methods

A trial to determine the best time to collect beetles from an established field population was conducted in spring 1996 at a site at Lincoln, Canterbury, on an established stand of *C. scoparius* where *B. villosus* infested approximately 45% of seeds. The site contained six 10 × 10-m blocks of even-aged mature *C. scoparius*, and each collection method was trialed in each block. On 16 September *C. scoparius* plants were classified as “pre-flowering”, as only a few flowers were present. On 3 occasions at different times of day (0800, 1200, and 1600 h) *C. scoparius* foliage was sampled for beetles in 2 randomly selected blocks using a Vortis® insect suction sampler for 5 min. Samples were collected from both flowering and non-flowering branches. On 2 and 15 October the flowering stage was estimated by recording the percentage of open flowers on branches selected at random along a transect. Samples were collected as on 16 September, except that non-flowering branches were not sampled.

A trial to determine the most efficient way of collecting beetles from an established field population was conducted at the same site. Three methods were trialed in spring when 50% of broom flowers had opened. All beetles collected were returned to the block from which they came, and sufficient time between trials was allowed so that beetles would disperse. As before, a Vortis® sampler was used to suck beetles from foliage, for a period of 5 min. The time taken to aspirate beetles from the collection container, and transfer them to a Perspex box, was also recorded. The second method involved beating flowering branches onto a 0.75 × 0.75 m cloth beating tray. Beetles were collected from the tray using an aspirator, and again transferred into a Perspex box. The total time taken to collect a minimum of 200 beetles was recorded, collecting from the tray as many times as was necessary, and completing the collection from the last tray once the 200th beetle had been aspirated. For the 3rd method, 5 flowering branches were cut from bushes, placed into a bag, and transferred to the laboratory where the branches were placed in a Perspex box 0.75 × 0.5 × 0.5 m. As they collected on the sides of the larger box, beetles were transferred to a smaller box using an aspirator. A 4th method was trialed in late summer when beetles were in pods. This method involved collecting branches bearing beetle-infested pods, and assumed that they would be transferred directly to a new site. Four branches bearing pods were cut from 4 different bushes from each block. From each branch 20 unopened pods were removed, and the numbers of beetles inside each was recorded. An estimate of the total number of beetles per branch was obtained by counting the number of pods per branch, and extrapolating the infestation rate from 20 pods.
Results

Numbers of beetles sampled at three different times of day were similar (F = 3.236, d.f. = 2, p = 0.087) so data were pooled for the 3 sample times. Results are presented in Table 3. Before the flowering season started, there were significantly more beetles on flowering plants. However, the number of beetles on flowers was significantly higher overall during the flowering period, and greatest at peak (50%) flowering. The most efficient method in terms of numbers of beetles collected per unit time, was seed collection in autumn, provided it is not necessary to separate the beetles from the plant material. The most efficient method in spring (including sorting samples) was beating (Table 4).

Table 3.
Numbers of *Bruchidius villosus* beetles collected from *Cytisus scoparius* flowers at three different flowering stages

<table>
<thead>
<tr>
<th>Flowering stage</th>
<th>Nature of sampled branches</th>
<th>Beetles collected / min. ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-flowering</td>
<td>non-flowering</td>
<td>0.37 ± 0.9</td>
</tr>
<tr>
<td>(only occasional flowers)</td>
<td>flowering</td>
<td>4.36 ± 1.01</td>
</tr>
<tr>
<td>10% flowering</td>
<td>flowering</td>
<td>27.5 ± 3.5</td>
</tr>
<tr>
<td>50% flowering</td>
<td>flowering</td>
<td>35.8 ± 7.7</td>
</tr>
</tbody>
</table>

Discussion

Early rearing methods for *B. villosus* were labor intensive, and because of difficulties encountered in inducing *C. scoparius* plants to produce flowers and pods under artificial light and in the absence of natural pollinators, populations were barely maintained. As rearing beetles through a generation in containment was difficult, in 1988 a strategy involving importing surface-sterilized seeds (Fowler and Speed 1989) was adopted. In this way beetles that had not been exposed to exotic plant material could be released from
containment directly they emerged from the treated seeds. However, once beetles had been re-phased to southern hemisphere seasons, effective rearing methods were developed that allowed large numbers of beetles to be released throughout New Zealand.

Rearing methods were developed simultaneously in the UK (Fowler and Speed 1989; Wilcox et al. 1991) and in New Zealand, each benefitting from the experience gained by the other. This was because low initial success rates in New Zealand meant that further importations of beetles were necessary, and it was several years before CABI Bioscience was able to supply adequate numbers to support large-scale rearing in New Zealand. As rearing progressed, efficiency increased. This was largely a consequence of the build-up of a wild population of beetles at the rearing site. The regime for maintaining beetles over winter was crucial to success. Beetle survival was high, and the “apartment blocks” were very low maintenance. We learned that it was better to bring beetles out of overwintering a few weeks before the optimal time for synchronizing them with the reproductive stage of _C. scoparius_ at late-flowering sites, allowing them to use the few early pods available, and adjust naturally to the phenology of the host plant, rather than suffer the high mortality of extended overwintering.

Two variations on release strategy were tested using beetle releases under our regional council-funded technology transfer program, and a farmer-led project in North Canterbury. Results from these trials gave us confidence in reducing the size of release (supporting research conducted by Memmott _et al._ (1996) that showed that making more releases of smaller numbers of individuals was more efficient that making fewer releases of larger founder populations). Also the results indicated that we could release beetles in late summer rather than in spring. This latter strategy allows beetles to be collected when they are most abundant, and when the collection method is most efficient, and when beetles can be transferred directly and easily to new sites.

Until beetles are abundant at release sites, it is important to search for them at the optimal time, when _C. scoparius_ is in full flower. Although intuitively it may be thought that beetles will be more easily found on the fewer early or late flowers (as beetles are attracted to flowers of _C. scoparius_), than at peak flowering, we have shown that beetles are more numerous on flowers at peak flowering. This is possibly because at times when flowers of _C. scoparius_ are not abundant, beetles forage on alternative pollen sources. In early spring substantial numbers of _B. villosus_ were found on flowers of gorse, _Ulex europaeus_ L. The methods trialed here were compared at only 1 site, where beetles infested over 50% of seeds. If beetle behaviour is density dependent, different results may be obtained when beetle densities are lower.

So far only 23 of the 98 releases of _B. villosus_ have established. Because it has often been difficult to recover beetles within the first 2 years of release, not all recent (1997-98) releases have yet been checked. Another factor that may cause a lower than expected establishment rate is that sites are not always checked at the optimal time for recovery of beetles, and therefore low populations may not be detected.

The work described in this paper is a result of interaction between researchers and users of biological control. Methods have been developed to increase the efficiency of rearing methods and enable initial populations of beetles to be released widely to as many clients as wanted them. Once field populations were established, methods were developed to allow users to harvest and redistribute beetles efficiently. Recommended procedures to manage _B. villosus_ and other weed biological control agents have been prepared as part of a handbook on weed biological control (Hayes 1999a).
Acknowledgements

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