Release strategies for the moth *Agonopterix ulicetella* in the biological control of *Ulex europaeus* in Chile

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Summary

The univoltine insect, *Agonopterix ulicetella*, was introduced into Chile from Hawaii and the UK in 1996 and 1997, respectively, for the biological control of gorse, *Ulex europaeus*. Release strategies to enable this agent to persist on gorse bushes were investigated. Five release sizes: 0, 2, 4, 8, 16 and 32 third-instar larvae, four times replicated, were made on gorse branches enclosed with a fine mesh sleeve. Over two seasons, agent population parameters and damage to the gorse branches were assessed. For *A. ulicetella*, the critical initial release size was eight larvae. The number of gorse shoots attacked by *A. ulicetella* was dependent on release size. The 8, 16 and 32 larval density levels resulted in a larger number of attacked shoots than did the lower larval densities.

Keywords: *Agonopterix ulicetella*, biological control, gorse, release strategies, *Ulex europaeus*.

Introduction

Gorse, *Ulex europaeus* L. (Fabaceae), is a perennial spiny shrub that originated in western Europe. It was introduced into Chile at the beginning of the 19th century and has since become a serious weed (Matthei 1995). The plant forms a dense, spiny, impenetrable scrub that gradually invades open ranges and competes with grass and forb species. Gorse is also a threat because it hinders the establishment and efficient management of exotic forest trees and constitutes a serious fire risk. In Chile, growth of gorse might reach a 30-fold increase per year (Norambuena 1995).

The gorse soft shoot moth, *Agonopterix ulicetella* (Stainton) (Lepidoptera: Oecophoridae), was first released in Chile in 1997, where it has one generation per year. The adult stage overwinters in the leaves, emerging during the spring to mate and lay eggs on the plant surface. Larvae feed and develop on leaves, particularly new growth, and then wander to pupate in the bushes. The new generation of adults emerge by early summer. This life cycle is similar to that reported by Hill *et al*. (1995).

One of the most critical problems following the introduction of weed biological control agents is the lack of experimental evidence relating to the optimal release strategy for successful colonization and establishment (Memmot *et al.* 1998). Currently, there are no theoretical grounds for making decisions about release size of biological control agents (Grevstad 1999a), and the optimal number of individuals to release at a site at any one time varies for different agent species. This number may depend on multiple factors (dispersal, ecoclimatic conditions, reproductive state, host phenology and quality etc.). Theoretical studies addressing the relationship between population size and persistence have indicated that, in general, persistence is predicted to be an increasing function of initial population size. Retrospective analyses of successful and unsuccessful deliberate introductions of biotic agents have also supported a positive correlation between initial colony size and establishment. The studies have compared establishment rates among species for which different numbers were released, rather than comparing the establishment rates of different sized releases within a species (Grevstad 1999b and references therein). As pointed out by Memmot *et al.* (1998) and Grevstad

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(1999b), in order to improve our ability to make decisions about release sizes in biological control, a strategy for propagation and distribution based on manipulative experiments should be developed for each control agent.

Improved release strategies may be needed for *A. ulicitella*. Apart from Chile, the moth has also been introduced into New Zealand and Hawaii for the biological control of gorse, but establishment success has been variable. In Hawaii, the insect is well established above 1000 m altitude (Markin *et al*. 1996) and is still producing noticeable damage (G. Markin and R. Hill, 2003, pers. comm.). In New Zealand, where it was first released in 1990, researchers had to develop a sex attractant to enable its recovery in the field (Suckling *et al*. 2000).

In Chile, *A. ulicetella* successfully overwintered in six of nine localities after its first release during the 1997/1998 season (Norambuena *et al*. 2000). Although larvae and feeding damage were observed at two of the original release sites during the past four years, population increases at these surviving sites has been slow (Norambuena, unpublished results). Difficulties in detecting *A. ulicetella* life stages or larval damage have resulted in farmers and forestry biocontrol supporters becoming sceptical about the usefulness of this agent. Furthermore, the moth's univoltine life cycle, which includes an obligate adult diapause, has made its propagation a rather slow process, making it difficult to decide on an optimal release strategy (i.e. how many individuals to release and when and how to release them). There has therefore been a need to investigate release strategies in order to improve the possibility of field establishment .

This paper presents the results of a field experiment with *A. ulicetella* to calculate the number of larvae that can be released in sleeve cages, as well as determining survival thresholds and feeding damage.

Materials and methods

Experiments were conducted in a field site located 20 km northeast of Temuco, Chile (38º41'S) from December 2000 to January 2003. An invading three-year-old gorse infestation of approximately 0.1 ha in size on an abandoned cultivated field constituted the study area. New gorse branches with stems from 0.7 to 1 cm in diameter and with 12 to 20 shoots per branch were selected from the edges of the gorse front for use as experimental plots. The total mean gorse shoot length per branch ranged from 441 to 468 cm in the release size treatments (Table 1).

Six releases sizes consisting of 0, 2, 4, 8, 16 and 32 third-instar larvae four times replicated were randomly made on gorse branches, making a grand total of 248 larvae released on 20 branches. The control releases (0 larvae) were used for determining whether natural *A. ulicetella* infestations had occurred and for assessing any treatment effects on gorse.

Branches were enclosed with a fine gauze sleeve 45 × 145 cm (length × diameter) in size and open at both ends. A conical wire structure of 70 × 40 cm served to fasten the sleeve over the branches. The whole structure was further affixed by hanging it from a supporting wire line located about 40 cm above the sleeves. Before the release, one end of the sleeve was secured around the branch with a plastic twist-tie. The other end, including the distal part of the branch, was also secured with a plastic twist-tie immediately after the release of the insects. The gauze sleeves were replaced annually.

The larvae used in the experiment originated from a hybrid population (UK/Portugal) introduced from Hilo, Hawaii. Initially, larvae were reared in the field on gorse plants enclosed in walk-in cages made of mesh fabric (2 × 2 × 2 m), similar to the cages described by Briese et al. (1996). In the laboratory, selected third-instar larvae were randomly assigned to each release size treatment and then transported to the field in ventilated plastic vials containing pieces of gorse shoots. The larvae were kept in a cool box until their release on a single day in late spring (19 December 2000). The releases were made by opening the vials and encouraging the larvae onto the growing gorse shoots. Larvae were spread around the shoots using a fine camel hair brush.

A. ulicetella population censuses and measurements of host plant parameters (shoot length, branch diameter, healthy and damaged shoots) were made at about one month, and one and two years after the releases. Counts

Table 1. Shoot length and stem diameter of gorse.

Release size treatments	Shoot length (cm)[a]		Branch diameter (cm)		
	Dec 2000 Mean (SD)	Dec 2002 Mean (SD)	Dec 2000 Mean	Dec 2001 Mean	Dec 2002 Mean
32 larvae	468 (91)	Na	0.88	1.28	1.3
16 larvae	441 (48)	Na	0.83	1.15	1.23
8 larvae	445 (57)	Na	0.88	1.13	1.25
4 larvae	446 (56)	1809 (912)	0.80	1.30	1.48
2 larvae	456 (62)	2420 (1515)	0.85	1.25	1.55
0 larvae	443 (49)	1557 (616)	0.78	1.25	1.33

[a] = total shoot length per branch.
Na = not assessed due to the presence of *A. ulicetella*.

of *A. ulicetella* developmental stages were made by searching inside the gauze sleeves and carefully examining all gorse shoots present on each branch, as well as the faeces and remaining host-plant material accumulated at the bottom of the sleeve. Any pupae present were returned to the sleeves. Data were square-root-transformed. Percentages of damaged shoot were arcsin-transformed before being subjected to a nonlinear regression analysis to estimate detectable feeding damage thresholds.

Results

Sampling effectiveness

The consistent recovery rates for larvae and pupae one month after the initial releases showed that there was no difference in sampling efficiency between the five release sizes. The risk that any variation in numbers of *A. ulicitella* found in different release-size treatments might be due to a sampling effect was therefore considered low (Table 2). Throughout the sampling period, there was no evidence of *A. ulicetella* developmental stages in the control plots. This, and the similar length of gorse shoots and diameter of gorse branches, in both control and infested treatments at the onset of the experiment (Table 1), satisfied the requirements for evaluating the influence of the release size on any detectable feeding damage on gorse and on the survival threshold of the agent.

Table 2. Recovery rate of *A. ulicetella* per treatment after one month.

Treatment	Recovery rate (SD)
32 larvae	0.64 (0.07)
16 larvae	0.56 (0.15)
8 larvae	0.56 (0.36)
4 larvae	0.75 (0.35)
2 larvae	0.63 (0.47)
0 larvae	0

Estimation of net reproductive rate

To calculate the estimated net reproductive rate/individual ($R = p/\theta$), a constant value (p) and the probability of recovering one individual present on the branch (θ) were calculated (see Memmot *et al*. 1998). The θ value was estimated by dividing the total number of recovered *A. ulicetella* one month after the initial release, corresponding to the lowest and highest release sizes (87 larvae), by the released number of larvae of these treatments (136 larvae). This resulted in an insect recovery rate of 0.639 (Table 1).

To estimate the p value, the initial number of released larvae and the realized number of insects found 13 months later were transformed to square-root to stabilize the variance and the data were then fitted to a linear regression with the equation $y = px$. The function line was forced through the origin so that the slope

of the line corresponded to square-root of p value $= 1.09$ ($r^2 = 0.62$). Back transformation yielded a value of $p = 1.04$. Therefore, the estimated net reproductive rate was $R = 1.63$ which means that the *A. ulicetella* released were able to replace themselves in the first year after the release. Regression analysis showed that the square root of the number of released larvae per branch explained 62% of the variation after 13 months in the field (Fig. 1.)

When the same model was applied to the data of *A. ulicetella* recovered after two years, (December 2002), but plotted against the realized number recovered the previous sampling date (January 2002), instead of using the original release sizes, the regression line explained only 53% of the variation in the dependent variable (Fig. 2). The net reproductive rate of the population during the second season resulting from p/θ ($p = 0.63$ and $\theta = 0.639$) was 0.99.
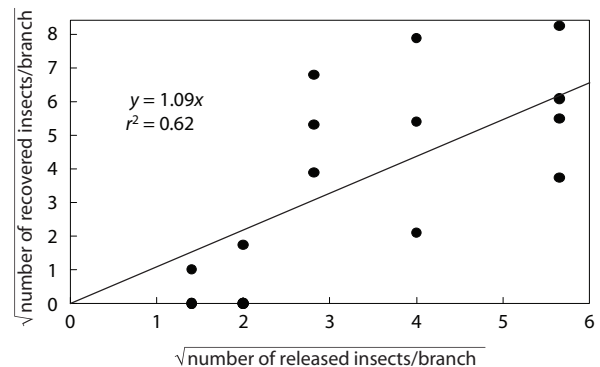

Figure 1. Relationship between the number of *A. ulicetella* recovered vs the number of larvae released one month earlier (both variables were square-root transformed).
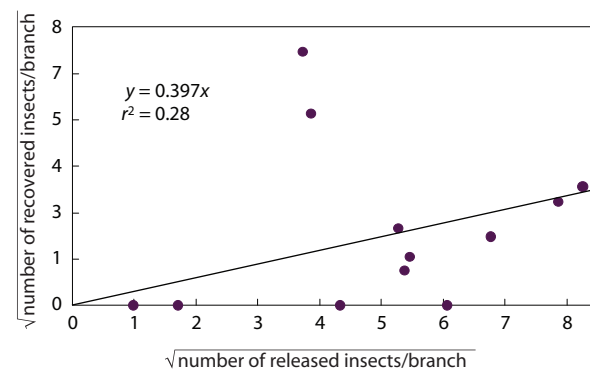

Figure 2. Relationship between the number of *A. ulicetella* recovered versus the number of larvae released one year earlier (both variables were square-root transformed).

The alternative method to estimate reproductive rates (Memmot *et al*. 1998) was used with each of the initial release sizes. The net reproductive rate one year after the releases indicated substantial differences between the two and four larvae releases size and the three highest initial larval release sizes. These increased

as the release sizes increased from 8 to 32 larvae (Fig. 3). This alternative method was also used to calculate the net reproductive rates of populations of 30, 37 and 37 *A. ulicetella*; populations that resulted from the initial release sizes of 8, 16 and 32 larvae, respectively. Recovered populations of the smaller release sizes were disregarded from the analysis as they did not produce any progeny after two years. The highest net reproductive rate ($R = 0.9$) resulted from the populations originating from initial release sizes of 32. The second highest rate ($R = 0.53$) resulted from the population originating from releases of eight larvae (Fig. 4).
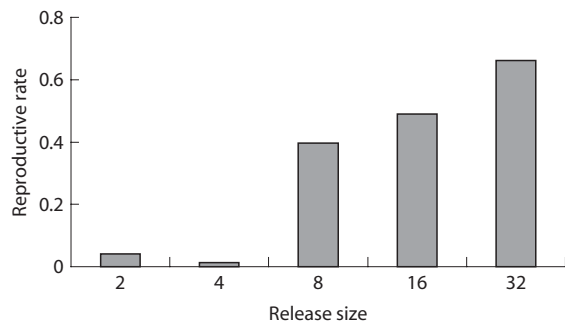

Figure 3. The relationship between reproductive rate of *A. ulicetella* and its release size after one gorse growth season.
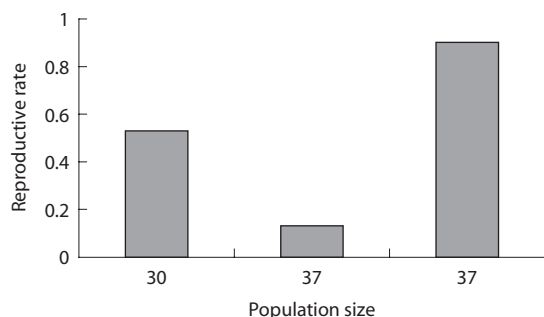

Figure 4. The relationship between reproductive rate of *A. ulicetella* and its release size after two years.

Colonisation patterns and release size

Colonization patterns of the 20 released populations are plotted in Figure 5. Population size varied markedly among the release sizes about one month after the initial release, ranging from 0 to 2, 1 to 4, 1 to 8, 7 to 12 and 18 to 22 for the 2, 4, 8, 16 and 32 larvae release sizes, respectively. All populations, except five belonging to the size releases of 2, 4 and 8 larvae, decreased in population size. One year after the initial releases, all populations of the 32 larvae release size treatments, and three populations out of four of the 16 and 8 release size treatments, increased in size. One population out of four of the four larvae release size treatment survived the first year before going extinct. Only one population of the release size of two larvae survived the first year. After two years, all populations decreased, excepting one of the 32 and one of the 8 larvae release sizes. Inter-

estingly, one population belonging to the release size of two larvae, which was undetected after one month, reappeared the following season, but became extinct during the second year.

Feeding damage

Shoot damage data obtained for the varying release sizes of *A. ulicetella* one year after the initial releases were best-fitted to a logistic function (Fig. 6, $R^2 = 0.88$). Although the number of shoots damaged by *A. ulicetella* was noticeable in all the release sizes treatments, it was substantially higher in the release size treatments of 8, 16 and 32 larvae than in the smaller size releases. No additional benefits in terms of feeding damage were noticed by releasing 16 and 32 larvae, compared with the treatment where 8 larvae were released. When data of the percentages of damaged shoots were arcsin transformed before plotting, this relationship was even stronger ($R^2 = 0.98$) and indicated that eight larvae were able to attack about 86% of gorse shoots.

After two years, the number of damaged gorse shoots were also best fitted to a logistic function (Fig. 7, $R^2 = 0.49$). No damaged shoots were detected on branches exposed to the two and four larvae release size treatments. Similarly to the previous year, the eight larvae release size treatment was sufficient to demonstrate detectable feeding damage on the gorse branches. When data for the number of shoots damaged were transformed, this relation was $R^2 = 0.58$, indicating that about 52% of gorse shoots were damaged with releases of eight larvae.

Discussion

The decrease in most of the release sizes treatments about one month after the initial release (Fig. 5), particularly in the highest release size treatments, may have resulted from manipulation of the larvae during the infestation process. This pattern was less strongly expressed in the smaller release sizes, perhaps because the larvae were handled more carefully. However, it is also possible that the larvae in the lower release size treatments were easier to find. Population decreases following the initial release of weed biological control agents have also been reported by Grevstad (1999b) who attributed this result to stress due to the host change (i.e. when the insects used in field experiments are obtained from laboratory rearing). In our experiments, *A. ulicitella* was retained in the laboratory for only a short period before release, so manipulation might have been a more important factor on the pattern of colonisation after one month rather than at later sampling dates.

None of the populations of the smallest releases sizes treatments (two and four larvae) survived the second year. Demographic stochasticity was a likely reason for the extinction of these populations, although an Allee effect may have played a role because preda-
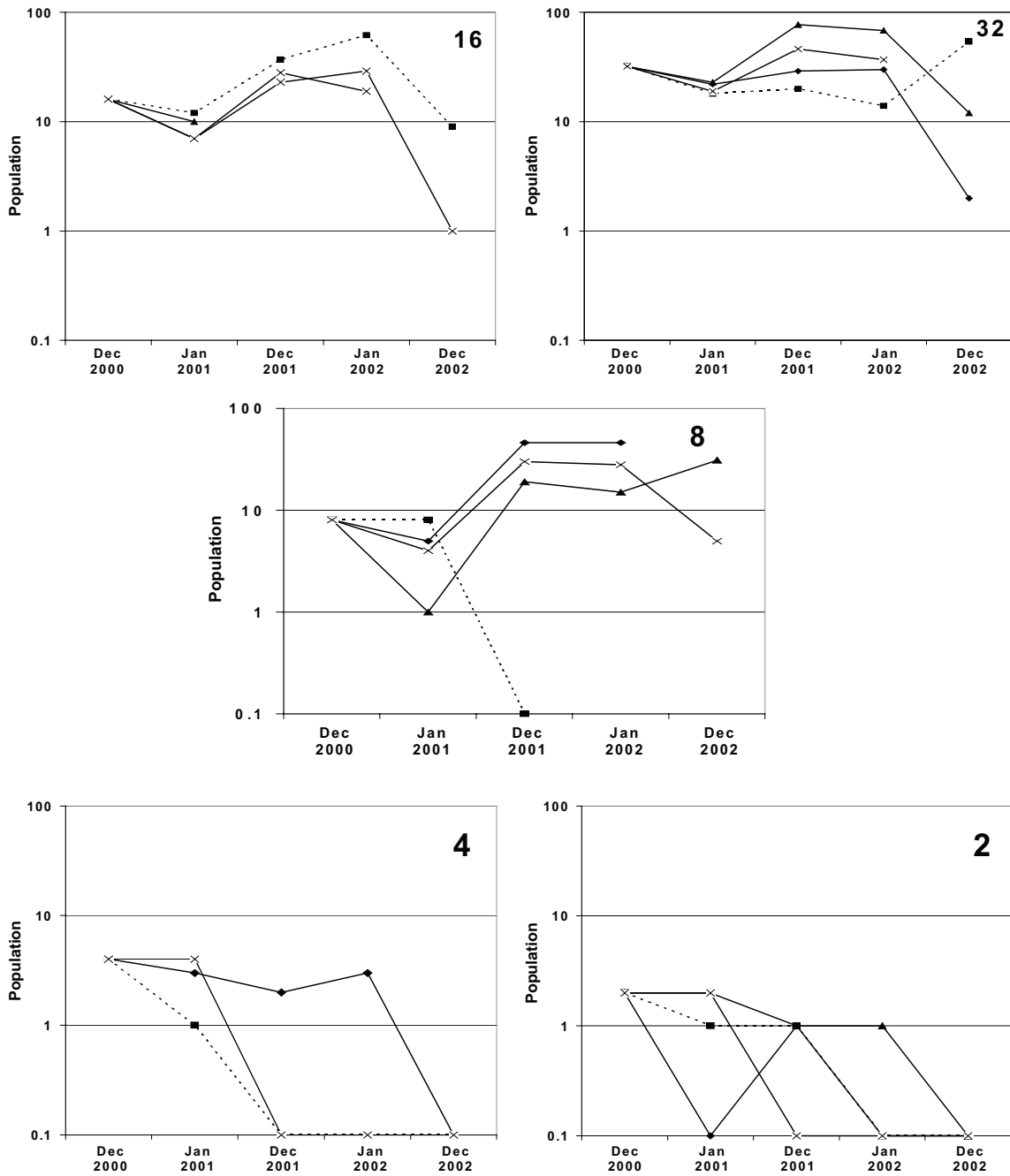
Figure 5. Numbers of *A. ulicetella* recovered at different sampling dates in each of the four replicates of the release size treatments.
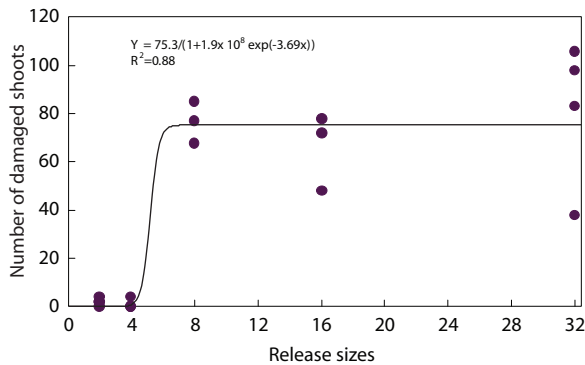

Figure 6. Numbers of shoots damaged by *A. ulicetella* in the release size treatments after one year.
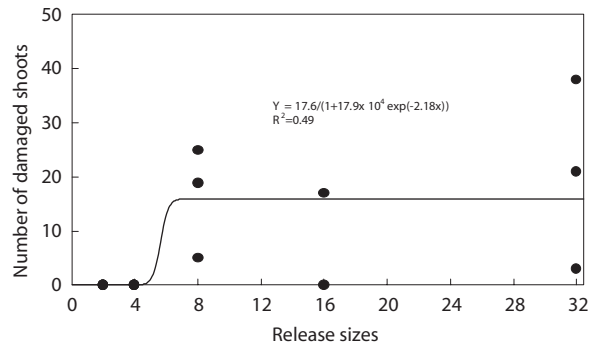
Figure 7. Numbers of shoots damaged by *A. ulicetella* in the release size treatments after two years.

tors (spiders and Carabidae larvae) were occasionally seen inside some of the sleeve cages. For instance, predation impact might have been less strong inside sleeve cages containing more *A. ulicetella* (larger release size treatments) than inside sleeve cages containing fewer larvae (smaller release size treatments) due to the strong defensive mechanisms of the fifth-instar larva, which, when disturbed, quickly moves backward inside a tunnel it builds with gorse spines. This behaviour may have favoured aggregation of *A. ulicetella* at more infested gorse branches and survival of sufficient individuals to ensure mating. Overall, populations of larger releases (8, 16 and 32 larvae) were clearly less likely to become extinct during the two-year study period than those originating from the smaller releases. The observed decline of some populations of the larger release sizes may have been due a differential shortage of the food resources provided by the host branch, as their shoots were highly damaged after one season (Fig. 6) and the sleeve cages prevented *A. ulicetella* from searching for a new food supply. This is coincident with the 0.99 net reproductive rate of the insect during the second season (which was calculated independently of the releases of two and four larvae) as compared with the 1.7 value obtained for the population originating from the initial release sizes during the first year.

Despite the decrease in larval numbers one month after the initial releases, a second generation of *A. ulicetella* was produced in all the release size treatments during the first year of the experiment, with survival rate increasing with release size (Figure 3). However, during the second year (Fig. 4) reproductive rates of populations originating from initial release sizes of 8 and 32 larvae were above 0.5 and 0.9, respectively, both of which were higher than populations originating from the 16 larvae release size. The lower survival rate of populations originated from the 16 larvae release size after two years might have occurred because a new generation was recorded in two of the three replicates that remained, and in one of these replicates only one larva was observed.

Thus, releases of eight third-instar larvae appear to be acceptable as a survival threshold of the control agent under sleeve cage conditions after one and two years. Furthermore, this release size was sufficient to demonstrate a detectable feeding damage of 88% of shoot damage after one year (Fig. 6), and of 49% (Fig. 8) after two years, from the onset of the experiment.

This experiment demonstrated that release size of *A ulicetella* did affect survival and had an impact on gorse shoot damage during two field seasons. Although releases of eight third-instar larvae appeared to be acceptable as the optimal release size for survival and detectable feeding damage, it cannot be assumed that this release size would lead to the field establishment of *A. ulicetella*, as the experiment was performed under

confinement. Even so, sleeve cage releases could be a useful technique for initiating the colonization of *A. ulicetella* as well as being used to demonstrate the potential impact of *A. ulicetella* on gorse to farmers.

The experimental results might also become useful in the future establishment of *A. ulicetella* as part of the current gorse biocontrol project in Australia, where importation of *A. ulicetella* will be considered on the basis of the outcome of host-specificity testing (Ireson *et al.* 2004, this volume).

Acknowledgements

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