Preliminary results of a survey on the role of arthropod rearing in classical weed biological control

R. De Clerck-Floate,[1] H.L. Hinz,[2] T. Heard,[3] M. Julien,[4] T. Wardill[5] and C. Cook[6]

Summary

The rearing of arthropods is an essential but sometimes neglected and underestimated part of a classical weed biological control programme. Success in rearing is usually a pre-requisite to conducting host-specificity tests, obtaining enough individuals for initial field release or, later, for large-scale implementation. Although most biological control researchers can list situations where agent development has been stopped or slowed due to rearing difficulties, failures seldom get reported in the literature, thus preventing us from gauging the extent and relevance of rearing issues. To rectify this, a questionnaire was developed to investigate the prevalence of rearing problems in weed biological control programmes and to classify their occurrence according to a list of variables (e.g. taxonomy, biological features, genetic issues and researcher/programme attributes). The questionnaire was sent to 80 researchers from eight countries; 65% responded, generating 79 useful responses. Results confirm that, of the challenges faced in programmes, rearing is the most prevalent (56% out of ten possible general problem categories). The most common rearing problems encountered were conditions that were not conducive to mating and/or oviposition (30% of reported arthropod cases) or development (22% of reported arthropod cases). Our results identify key areas for rearing improvement, thus contributing to increased weed biological control project successes.

Keywords: international survey, project challenges, rearing difficulties.

Introduction

The ability to rear arthropods for classical weed biological control programmes is a topic seldom formally addressed within our scientific discipline, yet it touches all stages of a programme and has the potential to seriously affect its progress and direction. Rearing can ensure a reliable source of arthropods for either host-specificity testing or initial field releases when agents are rare in their place of origin (Blossey *et al.*, 2000). Furthermore, because host-range testing relies on arthropod behaviours that also are required for rearing (e.g. mating, oviposition), the testing of these arthropods can be greatly hindered or prevented if they cannot be reared in a laboratory environment (Palmer, 1993; Marohasy, 1994; Klein, 1999). Sole reliance on field-collected arthropods from the country of origin for either host-specificity testing or field release is risky. For instance, events that eliminate a field population of arthropods used in testing could set back a programme for years, at great cost. This could be averted if a reared colony of the organism is available. There also is less control over factors that may influence host choice (e.g. prior host experience, readiness for host acceptance; Marohasy, 1998) when field-collected arthropods are used directly in host-specificity tests.

Once an arthropod has been given regulatory approval for release, the possession of a reared colony can greatly aid in achieving successful establishment. This is especially true for arthropods that may be susceptible

[1] Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, AB, Canada T1J 4B1.
[2] CABI Europe-Switzerland, Rue des Grillons 1, 2800, Delémont, Switzerland.
[3] CSIRO Entomology, 120 Meiers Rd, Indooroopilly 4068, Australia.
[4] CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier sur Lez, France.
[5] University of Sheffield, Department of Biomedical Science, Western Bank, Sheffield S10 2TN, UK.
[6] University of Queensland, School of Natural and Rural Systems Management, St. Lucia, QLD 4072, Australia.
Corresponding author: R. De Clerck-Floate <floater@agr.gc.ca>.

to Allee effects (e.g. ability to find mates), demographic stochasticity or catastrophic environmental events if released in small numbers (Hopper and Roush, 1993; Grevstad, 1999). It also is preferable for the sake of risk management to release agents from a reared colony of known taxonomic identity, purity and genetic source to reduce the possibility of unwanted inclusions such as other species or parasitized or diseased individuals. Although exemptions are possible, a regulatory condition of the Australian permitting system is that the agent must undergo a minimum of one generation in containment before release to avoid such hazards. Other countries, or laboratories within countries, may also attempt to follow this practice for precautionary reasons. Thus, the inability to rear an agent in the laboratory environment may present a direct impediment to its release.

The ability to rear weed biological control agents also influences the widespread implementation of successful agents. Typically, arthropods that are easy to rear and demonstrate rapid population increases under artificial conditions are the most amenable to mass production (Grossrieder and Keary, 2004). Compared to the mass production of entomophagous biological control agents for augmentative use (Thompson, 1999), the development of quality-controlled, mass rearing techniques for classical weed biological control arthropods is still in its infancy. However, recently, there has been a growing effort to experimentally develop methods for laboratory mass rearing of weed biological control agents on plants (Visalakshy and Krishnan, 2001) or artificial diet (Blossey *et al.* 2000; Wheeler and Zahniser, 2001; Goodman *et al.*, 2006; Raina *et al.*, 2006) or within scaled-up outdoor nurseries (Story *et al.*, 1994, 1996; Julien *et al.*, 1999; De Clerck-Floate *et al.*, 2007).

Although examples of failures or difficulties in arthropod rearing are well-recognized, these are seldom reported in the literature (however, see Palmer, 1993; Klein, 1999). The arthropods involved are either dropped from or reduced in priority on candidate lists, even if they show potential as effective agents. To determine the prevalence and types of rearing problems within the field of classical weed biological control, an international survey of researchers was conducted. This paper summarizes key, preliminary results and provides some perspective on how rearing can be managed to improve the science and implementation of weed biological control.

Materials and methods

A questionnaire on the role of arthropod rearing in classical weed biological control programmes was prepared through collaboration and piloted among six colleagues to ensure that the questions were clearly stated for the information requested. The questionnaire was divided into subsections: (1) general background on the researcher – name, location, years and type of experience in classical weed biological control; (2) programme-specific information (i.e. per weed) – duration of programme, number of arthropod species available on the weed in its place of origin, number of arthropod species tested, released and established, indication of avoidance of arthropod groups because of rearing issues, role of release size on arthropod agent establishment, role, prevalence and type of mass rearing in programmes, prevalence of monitoring for arthropod quality in reared colonies, evidence of genetic management during rearing (i.e. measures taken to reduce likelihood of inbreeding), a detailed list of all arthropod species either used or considered for biological control of the weed, with information on taxonomy, feeding guild and any problems encountered during development or consideration of the agent. The arthropod species-specific information became a large and key component of the data set and allowed analyses by family, order, feeding guild, 'general problems' encountered in the development of each agent (e.g. 'ability to rear'; Table 1), specific 'rearing problems', if they occurred (Table 2) and whether a species was rejected or given lower

Table 1. General problems encountered in classical weed biological control programmes and their frequency of occurrence.

General problems for weed biocontrol programmes	Count	n[a]	Frequency (%)
Ability to rear	246	439	56
Ability to establish agent in the field	72	362	20
Collecting sufficient specimens during exploration stage	85	444	19
Ability to elicit appropriate oviposition or feeding behaviours during host-specificity testing	50	411	12
Detecting/confirming establishment at release sites	35	322	11
Inadequate taxonomy	40	444	9
Ability to accurately document agent impact on the target weed	28	319	9
Obtaining/collecting sufficient agents for general distribution	27	321	8
Other	24	315	8
None	62	319	19

[a] 'n' for each problem varied depending on whether the programme stage allowed for encounter of the problem, and thus the inclusion of appropriate cases in the calculation of frequency.

Table 2. Specific problems encountered when rearing arthropods for classical weed biological control and their frequency of occurrence (n = 246). Note that more than one problem could arise per arthropod case that was listed.

Rearing problems	Count	Frequency (%)
Experimental conditions not conducive to mating and/or oviposition	74	30
Experimental conditions not conducive to normal development	53	22
Long life cycle	52	21
Obtaining appropriate phenological stage of host plant for rearing	51	21
Unknown biology or life cycle	49	20
Field collection of enough individuals to start a laboratory colony	44	18
Host plant nutrition/quality	34	14
Incompatibility with host plant	33	13
Mortality during storage of diapausing arthropods	28	11
Synchronization between northern and southern hemispheres	27	11
Presence of parasitoids	26	11
Presence of pathogens	20	8
Failure to break diapause	19	8
Difficulties with artificial rearing medium	7	3
Inadequate labour or facilities	6	2
Presence of predators	4	2
Inbreeding or genetic adaptation to laboratory conditions reducing quality of colony	4	2
Presence and possible effects of endosymbionts	4	2
Mutualist needed	3	1
Difficulties in distinguishing sexes	3	1
Other	4	2

Table 3. The stages in classical weed biological control programmes at which rearing problems occurred and the number and frequency of occurrence for each stage (n = 85).

Project stage	Count	Frequency (%)
(A) Exploration	16	19
(B) Host-specificity testing	43	51
(C) Early release and monitoring for establishment	20	23
(D) Mass production	4	5
(E) General distribution of agents	2	2
(F) Monitoring for impact by agents	0	0

priority at any point because of rearing problems. Only summary results from the survey are presented in this paper due to the large volume of information that was obtained. A journal publication presenting the broader results is in preparation.

A total of 80 researchers from eight countries were surveyed. Respondents were asked to complete a separate questionnaire for each weed species with which they had been involved and to only answer questions pertinent to their experience within programmes, as identified by stage (Table 3). Programme stage was taken into account during determination of the prevalence of various general problems or rearing problems by including these data for summary or analysis only if the researcher could have conceivably encountered the problem(s) at their programme stage(s). This controlled for any bias due to stage of programme during data summary. For example, General Problem, 'Ability to accurately document agent impact' (Table 1) would only be encountered at stage F of a programme (Table 3); thus, only agents in programmes that had reached stage F were included in calculations involving this problem.

Results and discussion

General results

A total of 52 researchers (65%) responded to the survey, generating 84 questionnaires, which represent the majority of active weed biological control programmes worldwide. The countries and number of researchers based in each were Australia (16), South Africa (9), USA (9), Switzerland (6), New Zealand (5), Canada (4), France (2) and Argentina (1). The average experience in weed biological control of the researchers was about 19 years, and the cumulative experience by the respondents was 987 years. Most of the researchers were based in the introduced range of the plant (54%), some in the native range (21%), while others worked in both (25%).

Of the 79 questionnaires that were included in the analyses (five were not completed properly and were excluded), the median duration of the weed programmes was 17 years. All programmes had completed stage A, exploration and were at least in stage B, host-specificity testing (see Table 3 for a list of stages). The majority of programmes in the introduced range had reached the final stage F (monitoring for impact). In 65% of cases, respondents reported work on a unique target weed, while in 35% of cases, the same species was reported from another country (i.e. work within native vs introduced ranges or two or more countries working on the same weed) or at a different programme stage. Specifically, the same weed was reported twice in eight cases and three times in four cases.

The analysed responses covered a total of 64 weed species located in various climatic zones including; temperate (32 species), temperate/subtropical (5), subtropical (11), subtropical/tropical (9), tropical (6) or temperate/subtropical/tropical (one aquatic species). The majority of target weeds were perennials (52 species) with a few biennials (7) or annuals (5). The number of herbivorous arthropod species occurring on a weed in its area of origin was estimated through the literature, field surveys or both by respondents for 45 of the target weeds. The average number of herbivores was 120 but generally increased from temperate to tropical zones (Fig. 1), which is an expected trend based on studies of arthropod diversity along latitudinal gradients (Price, 1997).

Information on a total of 384 arthropod species (nine eriophyoid mites and 375 insect species) were recorded in the questionnaires. The insects were from 6 orders and 66 families. A few of the arthropods were used for more than one weed target, resulting in a total of 444 cases for data analysis.

Incidence of rearing problems

Respondents reported many general problems in the development of arthropod agents for weed biological control; by far, the most common was 'ability to rear' with 56% of the arthropod cases listed in questionnaires (Table 1). Other common problems, such as 'collecting sufficient specimens during exploration stage' and 'ability to establish agent in the field', may also be related to the ability to rear, as it is

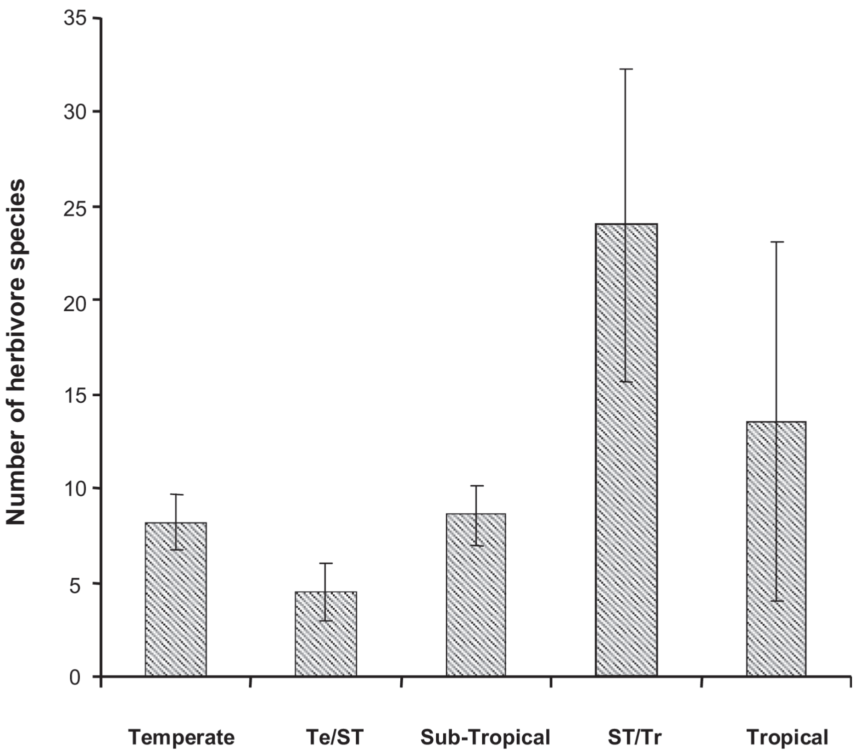

Figure 1. Mean number (±SE) of herbivore arthropod species estimated (from field surveys, the literature or both) to occur on a target weed species in its area of origin according to climatic zone. *Te/ST* Temperate/subtropical, *ST/Tr* subtropical/tropical.

suspected that they may not have attained this level of relevance if rearing were easy. For instance, there is evidence that releases at more sites and/or with larger numbers of individuals per release in a geographic area improves establishment success (Hopper and Roush, 1993; Memmott *et al.*, 1998; Grevstad, 1999). It has also been acknowledged that difficulties in rearing may jeopardize agent establishment where there are known hazards to small, founding, field populations (Spafford Jacob *et al.*, 2007). In our questionnaire, 73% (174/237) of answered cases indicated that there were examples within programmes where it was suspected that failure to establish may have been due to small release size.

Specific rearing problems experienced by researchers can be grouped into several categories. The most common were conditions that were not conducive to mating and/or oviposition or normal development of the immature stages (Table 2). Often, appropriate artificial light and temperature conditions must be experimentally identified to induce arthropod behaviours required for successful rearing (Baars, 2001). In addition, very common were problems due to long or unknown life cycles or unknown biology. Issues related to the host plant such as nutrition, quality, incompatibility or providing the correct phenological stage were very common. Obtaining enough individuals to start a colony was a common problem as were problems with synchronization and diapause. Issues associated with organisms such as parasitoids, pathogens, predators, endosymbionts and mutualists were reported less frequently. In some situations, arthropods reared under laboratory conditions simply did not thrive for obscure reasons, which require further investigation (Hoffmann, 1988). For instance, among respondents to this survey, only 2% of cases had ‘inbreeding or genetic adaptation to laboratory conditions reducing quality of colony’ listed as a rearing problem (Table 2); yet, when they were specifically asked whether they monitor for arthropod quality (e.g., fecundity, fertility) during rearing, only 39% (170/430) of cases were affirmative, and in 44% (190/430) of cases, agents were not monitored at all. This lack of quality control clearly indicates a reduced possibility of identifying genetic problems in rearing, even though they are known to occur (Torres *et al.*, 1991; Wardill *et al.*, 2004). Changes in fecundity or fertility also may indicate the effect of endosymbionts such as *Wolbachia* spp., which are seldom considered in biocontrol programmes (Floate *et al.*, 2006).

The stage in a project in which an arthropod was rejected or given lower priority because of rearing problems was recorded for only 85 cases out of 444 (Table 3). Despite the poor response to this question, the results still show that when decisions are clearly made due to rearing difficulties, most rejections or priority adjustments occur during the host-specificity testing stage. Of note is that 20 species were rejected at the release stage and four at the mass-production stage. These 24 cases may represent very costly situations, as large programme expenses were likely already incurred at the time of rejection.

Rejection or priority adjustment also occurred during ‘exploration’ when rearing is required for identification, biology and life-history studies and sometimes preliminary host-range tests (Table 3). After the survey, it occurred to us that there may have been a blurred boundary between exploration and host testing, thus inflating the relevance of the host-specificity testing stage as a time when rearing issues surfaced. For instance, if exploration were conducted through quick, infrequent visits to the place of weed origin by remotely situated researchers, rather than from a more permanent base located in the place of origin, then all work conducted in the recipient country may have been grouped under ‘host testing’. This ability to clearly distinguish the boundary between these two stages may be a limitation of the survey.

Importantly, identification of rearing difficulties in the early stages of a programme allows for either the deployment of resources to develop needed rearing techniques or the rapid adjustment of programme goals, so that resources can instead be allocated to candidates that are easier to rear (Hoffmann, 1988). Although details on the taxonomic affiliations of difficult-to-rear arthropods will be forthcoming in a future publication, 27% (104/379) of answered cases in our survey indicated that certain taxonomic groups or feeding guilds were initially either avoided or given lower priority due to perceived potential rearing problems. Interestingly, there was a positive linear relationship between the estimated number of species feeding on a weed in its place of origin and the number of agents tested by researchers ($R^2 = 0.605$, $P = <0.001$, $n = 364$). However, there was no correlation between the estimated number of species and the incidence of rearing difficulties ($R^2 = 0.003$, $P = 0.287$, $n = 381$). Thus, there was no indication that, if researchers have more insects to choose from as potential agents, they tend to test those that are easier to rear, or it could quite simply mean that we are poor at predicting at the start of a programme which arthropods will be easiest to rear.

Conclusions

The preliminary results of our survey indicate that arthropod rearing issues are perceived to be important among classical weed biological control researchers. They have a very important impact in the development of successful agents and therefore on the outcome of programmes. Despite this, there is ample opportunity to improve rearing efforts and methodology.

Where possible, we recommend that rearing be incorporated into the exploration stage of programmes, as suggested by Hoffmann (1988), or as very early studies in the recipient country in cases where exploration is practiced as quick visits for arthropod collection by remote researchers. Should rearing difficulties occur, emphasis could then be placed on developing laboratory-based rearing techniques. Only then should otherwise promising species with apparently insoluble rearing problems be given lower priority or abandoned as candidate agents before major costs are incurred. The fact that 27% of respondents avoid taxonomic groups that have perceived potential rearing problems may be good for immediate resource management. However, unless species in the 'troublesome' groups are studied, this avoidance will not lead to improved rearing capability, and potentially useful agents may be rejected prematurely.

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