IMPORTATION TO NEW ZEALAND QUARANTINE OF A CANDIDATE BIOLOGICAL CONTROL AGENT OF CLOVER ROOT WEEVIL

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ABSTRACT

Several candidate biological control agents of *Sitona lepidus* have been identified since a search commenced in 1997. Interestingly, *Microctonus aethiopoides* from Europe is a much more effective parasitoid of *S. lepidus* than the *M. aethiopoides* ecotype already established in New Zealand. To assess further the suitability of the European *M. aethiopoides* for biological control of *S. lepidus*, 1599 infected *S. lepidus* were shipped to New Zealand quarantine during late 2000. These yielded 267 parasitoid pupae from which 204 adult parasitoids were reared. This material was obtained from a wide geographical range in Europe and has been used to establish cultures in New Zealand quarantine based on genetic material from France, England, Norway, Finland, Romania, Ireland, Scotland, Italy and Wales. This contribution presents an overview of the work associated with the importation of the parasitoids and the effort now being made to maintain genetic diversity. Planned research is also discussed.

Keywords: clover root weevil, biological control, *Microctonus aethiopoides*, *Sitona lepidus*, *Trifolium repens*.

INTRODUCTION

Sitona lepidus was first recorded in Waikato, New Zealand, in March 1996 (Barratt et al. 1996) and has since spread at ca 35 km/year (Willoughby & Addison 1997a). Surveys have revealed extremely high densities both of adult weevils feeding on white clover foliage and of larvae on plant rhizobial nodules and roots. The associated reduction in plant vigour and nitrogen fixation has resulted in *S. lepidus* becoming recognised as a serious threat to New Zealand's competitive edge in pastoral production (Willoughby & Addison 1997b). In the last 20 years, the lucerne weevil *Sitona discoideus* and the Argentine stem weevil *Listronotus bonariensis* have been usefully suppressed by introductions of *M. aethiopoides* and *Microctonus hyperodae* respectively (Goldson et al. 1990; Goldson et al. 1998). Thus, an early response to the discovery of *S. lepidus* was to test whether either of these parasitoids would be useful against it. However, neither showed any potential for control of the weevil (Barratt et al. 1997).

A range of initiatives has been established to attempt to offset the threat posed by *S. lepidus*. One of these has been the implementation of a comprehensive effort to find suitable biological control agents. Preliminary work and searches conducted in 1998 and 1999 indicated that such agents are most likely to be found in Europe (Phillips et al. 2000; Goldson et al. 2001a). In the European summer of 2000, a major effort was made to identify and collect the most promising *S. lepidus* natural enemies from a wide range of locations. As it happened, *M. aethiopoides* was consistently found to be the best option (Goldson et al. 2001b; C.B. Phillips, unpubl. data) in spite of the converse observation of Barratt et al. (1997) that the New Zealand ecotype was ineffective. Indeed,

New Zealand Plant Protection 54:147-151 (2001)

this difference has highlighted the importance of thoroughly exploring intraspecific variation in biological control agents (Goldson et al. 1997).

This work resulted in ca 8500 *S. lepidus* being collected from 15 locations in 11 European countries. The parasitoids were reared out and identified, initially by AgResearch staff based at the USDA's European Biological Control Laboratory in Montpellier, France, and later at the Institute of Grassland and Environmental Research at North Wyke, Devon. All ecotypes of *M. aethiopoides* were found to develop readily in both British and New Zealand populations of *S. lepidus* (Goldson et al. 2001b; C.B. Phillips, unpubl. data).

Given the promising performance of *M. aethiopoides* collected in Europe, populations were shipped from North Wyke to New Zealand containment for culturing and analysis. This contribution reports on the forwarding of such material to New Zealand, how its genetic diversity is being maintained and the way in which it is being evaluated as a *S. lepidus* biological control agent.

METHODS AND MATERIALS

Shipments

Shipment of weevils within Europe was by post, using supplied pre-addressed 'bubble' envelopes and 60 mm x 20 mm vials with gauze tops into which the weevils were placed.

Four consignments of live parasitised weevils were forwarded to New Zealand by airfreight. Because the freight-forwarding companies were reluctant to transport 'live animals', it was easier to send the material directly using separate airway bills, thereby avoiding the use of the freight-forwarding companies. Effectively, this was via direct negotiation with Air New Zealand.

Each of the four shipments from North Wyke comprised up to eight 60 mm x 20 mm vials each containing up to 100 weevils along with a 40 mm x 50 mm corrugated cardboard strip and 4-5 trifoliate leaves of white clover. These vials were then packed into 244 mm x 196 mm x 106 mm (depth) polystyrene boxes with damp tissue placed on top. Each box was then securely taped before being sealed inside a pre-addressed and suitably labelled cardboard box.

Containment facilities

M. aethiopoides populations were transported to New Zealand as larvae in British and/or New Zealand *S. lepidus*. Given that a CSIRO-sourced population of *M. aethiopoides* was introduced into New Zealand in 1982 (Stufkens et al. 1987), the New Zealand Ministry of Agriculture and Forestry Quarantine Service was able to give permission for the additional live, conspecific material to be maintained in New Zealand containment facilities. Initially, the material which consisted of parasitised weevils from both Europe and New Zealand was imported to the Landcare Research transitional and containment facility at Lincoln. Here the parasitoids were reared to the adult stage whereafter they were used to infect Waikato-collected *S. lepidus*. After obtaining permission from MAF Quarantine, the parasitoid cultures were transferred to an AgResearch containment facility. Full attention to security has been maintained throughout as *S. lepidus* has yet to be found in Canterbury and any escapes could result in establishment of the weevil in the Lincoln district.

Maintenance of *M. aethiopoides* cultures and their genetic diversity

The primary aims of ensuing work in containment at Lincoln are to maintain each geographic population separately and to minimise losses of genetic diversity. This has demanded the ongoing development of a systematic rearing system specifically designed to minimise inbreeding, selection for laboratory fitness, and inadvertent loss of genetic diversity through extinction. Initially, depending on availability, up to four male/female pairs of *M. aethiopoides* from each geographical location were used to establish each population in culture. Thereafter, sibling mating was avoided whenever possible by allowing female parasitoids to mate only with males from another pair within their 'ecotype' (or 'hybrid') group.

Broadly, the method of rearing *M. aethiopoides* on *S. lepidus* is similar to that described for rearing *M. aethiopoides* on *S. discoideus* (Goldson et al. 1990). Newly-emerged

(<24 h) female *M. aethiopoulos* parasitoids are mated with single males in 60 mm diameter vials. The mated females are then individually confined for 48–72 h at 20°C in 220 mm x 130 mm x 75 mm cages with 40–60 Waikato-collected *S. lepidus*. The parasitoids are also provided with 5 mm squares of filter paper soaked in honey solution along with two or three white clover plants grown in 60 mm x 25 mm diameter soil plugs and sealed in 50 mm x 100 mm polythene bags. After removal of the parasitoid, the weevils are maintained for ca 3 weeks at 20°C. Their cages are fitted with a 0.5 mm gauze floor and positioned over a lower chamber into which are placed strips of paper towel. This allows the emergent prepupal parasitoids to drop through the gauze floor and pupate in the paper strips. The pupae are then transferred to 90 mm diameter Petri dishes along with a moistened dental wick until adult eclosion. Petri dishes are checked daily for newly-emerged parasitoids to reduce the possibility of sibling mating.

All biological material has been preserved either in alcohol or at –40°C for further research.

RESULTS AND DISCUSSION

The details relating to the material shipped to New Zealand are summarised in Table 1. The mean mortality level across these shipments was 19% and reflected the difficulty encountered in transporting live material from Europe to New Zealand. Nonetheless, by November 2000, six ‘ecotypes’ of *M. aethiopoulos* had been established in containment at Lincoln. These were from Aberystwyth (Wales), Athenry (Ireland), Heggenes (Norway), Mikkeli (Finland), Montpellier (France) and North Wyke (England). In addition, ‘hybrids’ were established from North Wyke/Brasov (Romania), Mikkeli/Pordenone (Italy), Mikkeli/Wigtown (Scotland) and Montpellier/Nancy (France) (Fig. 1). The mean level of parasitism of *S. lepidus* by *M. aethiopoulos* in the field in Europe, as determined by maintaining weevils in cages and monitoring the emergence of parasitoid pupae, was ca 2%. The higher levels of parasitism (12% – 29%) suggested by the data in Table 1 occurred because most *M. aethiopoulos* populations were reared for at least one generation in the laboratory at North Wyke before being imported to New Zealand quarantine.

TABLE 1: Details of shipments of *Microctonus aethiopoulos* forwarded from North Wyke to containment facilities at Lincoln, New Zealand.

Date received in New Zealand	Total number of weevils	Percent mortality	Total parasitoid pupae	Total parasitoid adults reared out from pupae
19 September 2000	249	15	73	58
25 September 2000	637	13	105	87
21 October 2000	403	31	53	39
11 November 2000	310	14	36	20
Total	1599		267	204

Ongoing maintenance of genetic diversity within the cultured populations has proved challenging due to: a) unpredictable and occasionally very low numbers of parasitoid progeny arising from exposures of parasitoids to *S. lepidus* in cages; b) highly variable sex ratios of the parasitoid progeny; c) difficulties in ensuring that female parasitoids are mated; and d) sporadic troubles obtaining sufficient *S. lepidus* from Waikato during wet weather when collecting is not possible. Due to these problems, bottlenecks have occurred in the culture. By May 2001, each population could be traced back to the following numbers of parental pairs: Aberystwyth (Wales) 2; Athenry (Ireland) 1, Heggenes (Norway) 3, Mikkeli (Finland) 1, Montpellier (France) 2, North Wyke (England) 3, North Wyke/Brasov (Romania) 2, Mikkeli/Pordenone (Italy) 2, Mikkeli/Wigtown (Scotland) 1, and Montpellier/Nancy (France) 3.

Refinements to the culturing methods are aimed at reducing the risk of losing further genetic stock. The culture is currently being used to provide material for analysis of the ecotypic variation of European populations of *M. aethioides* as it relates to their potential to act as control agents for *S. lepidus*. This includes investigation of fecundity, longevity, mating behaviour, attack rates, development rates, host range testing and, tolerance to *S. lepidus* immune responses. DNA-based investigation into the genetic structure of the European and New Zealand populations of *M. aethioides* and *S. lepidus* has also commenced.

FIGURE 1: Collection sites of *Microctonus aethioides* populations that were put into New Zealand quarantine between September and November 2000.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contributions made at North Wyke by Pip Gerard (AgResearch Ruakura) and Chris Galbraith (AgResearch Lincoln). Scott Hardwick, Birgitta Harens, Derrick Wilson, Fiona Kettlewell, Tina Eden and Bruce Willoughby from AgResearch, Ruakura, generously provided live *S. lepidus* for experiments in Montpellier and North Wyke, as well as for maintaining the *M. aethioides* culture at Lincoln. Simon Kelly, AgResearch, Lincoln, provided expert help in maintaining the parasitoid culture. The collection of *M. aethioides* from a wide range of European locations was achieved through the invaluable assistance of IGER at North Wyke, the USDA (EBCL) at Montpellier, and COST collaborators throughout Europe. Funding

was gratefully obtained from diverse sources including the Foundation for Research, Science and Technology, the Combined Pastoral Producer Boards, the Lincoln University Foundation, AGMARDT, the C. Alma Baker Trust and NZ Grasslands Association.

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