RATIONALE FOR RELEASE OF THE IRISH STRAIN OF *MICROCTONUS AETHIOPOIDES* FOR BIOCONTROL OF CLOVER ROOT WEEVIL

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ABSTRACT

A European biotype of *Microctonus aethiopoides* was identified as the best candidate biocontrol agent for *Sitona lepidus*, a serious New Zealand pasture pest. A Moroccan biotype was already present throughout the country and hence there was no requirement to obtain Hazardous Substances and New Organisms (HSNO) Act approval to release new biotypes. However, as research had shown mating between the two biotypes produced hybrids with poor efficacy against target hosts, and that the Moroccan biotype attacked several native weevil genera, serious reservations were held about introducing the European biotype. Concerns were overcome with the identification of a parthenogenetic strain of European *M. aethiopoides* from Ireland, which has little risk of hybridisation, and a narrower host range than the Moroccan biotype. Following regulation of *M. aethiopoides* as a risk species, this strain was considered to be a new organism. Approval was sought and gained under the HSNO Act to release the strain.

Keywords: *Microctonus aethiopoides*, *Sitona lepidus*, Environmental Risk Management Authority, biocontrol.

INTRODUCTION

When clover root weevil (CRW) (*Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae)) was first discovered in 1996, it was anticipated that two weevil parasitoids already present in New Zealand pastures, namely the braconids *Microctonus aethiopoides* Loan and *M. hyperodae* Loan, might provide at least partial control of CRW (Barratt et al. 1996). At that time the Moroccan biotype of *M. aethiopoides*, introduced in 1982 (Stufkens et al. 1987), was providing effective control for the lucerne pest *Sitona discoideus* Gyllenhal (Goldson et al. 2005), and a single specimen of *M. hyperodae* had been reared from CRW collected from the Ruakura Research Centre dairy farm (Barratt et al. 1996) where this parasitoid had been released in 1991. However, this hope was unfounded. Less than ten *M. aethiopoides* were reared from field collections over the following 10 years, and CRW rapidly became one of the most damaging clover pests found in New Zealand. Furthermore, laboratory tests confirmed that neither the Moroccan biotype of *M. aethiopoides* nor *M. hyperodae* were effective against CRW (Barratt et al. 1997b).

White clover (*Trifolium repens* L.) is a high value component of most pastures in New Zealand (Caradus et al. 1996). With a potential annual direct cost of the pest estimated

at $300 million a year (Barlow & Goldson 2002), the pastoral industry looked to the research community for solutions. With in-depth knowledge of weevil biology and control, AgResearch entomologists advocated the introduction of a biocontrol agent as a potential long-term solution to CRW that could be implemented without changing existing on-farm management systems.

SUMMARY OF THE SEARCH FOR A BIOLOGICAL CONTROL AGENT

The project commenced in 1998, initially in collaboration with CABI Bioscience Centre for exploration in Switzerland, and with the University of California, Berkley, USA, for searches in California and Oregon (Phillips et al. 2000). Progress was very slow until the European exploration was expanded with AgResearch entomologists setting up temporary research bases in France in 1999-2000 (Phillips et al. 2002) and subsequently in England in 2000 (Goldson et al. 2004). Collaboration with researchers undertaking comparative white clover field trials across Europe greatly facilitated the search, with weevil samples being forwarded from Italy, Romania, France, Netherlands, Norway, Finland and Sweden, and further collections undertaken by AgResearch staff in Ireland, Wales, England and Scotland (Goldson et al. 2004).

The parasitoids found were the braconids *M. aethiopoides,* an unidentified *Allurus* sp., *Allurus lituratus* Haliday and *Perilitus* sp., and the tachanid *Microsoma exiguum* Meigen (Goldson et al. 2004). Of these, *M. aethiopoides* was by far the most commonly recovered, both within and between countries, and had the highest rates of parasitism in laboratory tests compared to the other parasitoids recovered (Phillips et al. 2002; Goldson et al. 2004). In contrast to the Moroccan biotype of *M. aethiopoides* introduced to control *S. discoideus*, the European biotype showed excellent efficacy against New Zealand CRW. For these reasons the European *M. aethiopoides* was selected for further investigation.

Cultures of the European biotype from six countries were established in quarantine in New Zealand in late 2000 and research commenced to investigate suitability for introduction (Goldson et al. 2001). It was found that hybridisation between the European and Moroccan biotypes led to significant reductions in efficacy of *M. aethiopoides* against both CRW and *S. discoideus* (Goldson et al. 2003*).* This was a major set back but was overcome with the serendipitous discovery of an all-female strain of the European biotype in Ireland that reproduced by thelytokous parthenogeneticity and was effective against CRW (Goldson et al. 2005). *Microctonus aethiopoides* reared from subsequent collections throughout Ireland have all been parthenogenetic and molecular techniques have shown that this strain can be distinguished from other strains within the European biotype (Vink et al. 2003).

SUMMARY OF EFFICACY AND HOST TESTING

The Moroccan biotype of *M. aethiopoides* parasitises a number of indigenous and exotic non-target genera in the field (Barratt 2004), especially broad-nosed weevils in the subfamily Entiminae (subfamily classification of Leschen et al. 2003). While there is no evidence of an adverse impact on these weevil populations, the knowledge of this unexpected wide host range contributed to increased concern in New Zealand about the potential impacts of all new biological control agents on non-target species (e.g. Barratt et al. 2000), let alone a new *M. aethiopoides* biotype.

The host range testing of the Irish strain is summarised by Goldson et al. (2005). Representatives of the weevil genera attacked by the Moroccan biotype were tested in a series of no-choice and choice tests. It was found that the Irish strain was more selective and, with the exception of *Nicaeana cervina* Broun*,* had lower parasitism rates than its Moroccan counterpart. It is possible that the difference in attack rates may be associated with the virus-like particles which are present in Moroccan (Barratt et al. 1999) but not the Irish *M. aethiopoides* (Barratt et al. 2006). These particles may be important in the

suppression of the host weevil's immune defence system and allow a wider host range. Of the species tested, only *Irenimus aequalis* Broun and *N. cervina* are considered likely to act as hosts to the new strain, and both species are preferred less than CRW by Irish *M. aethiopoides* and had higher levels of non-viable parasitoid larvae.

In the host range studies, attack rates of the Irish biotype of *M. aethiopoides* on CRW averaged over 70% (Goldson et al. 2005). Comparative levels of 67% parasitism were achieved during similar laboratory testing of *M. hyperodae* against *Listronotus bonariensis* (Kuschel) prior to release (Barratt et al. 1997a) and this *Microctonus* species subsequently reduced host weevil populations in the field (e.g. Goldson et al. 1998).

HSNO ACT APPROVAL PROCESS

Under the Hazardous Substances and New Organisms (HSNO) Act 1996 a number of criteria define a new organism, the most relevant being an "*An organism belonging to a species that was not present in New Zealand immediately before 29 July 1998"*. With the Moroccan biotype already established in New Zealand, there was no need to obtain a HSNO Act approval to release the Irish strain. However, concerns about possible hybridization and known differences in host range suggested that a cautious approach should be taken. Because of this concern, at AgResearch's request, in December 2004 the Minister for the Environment prescribed *M. aethiopoides* to be a 'risk species'. As a result all strains, with the exception of the Moroccan biotype, are considered to be 'new organisms' and as such require a HSNO Act approval. HSNO Act approvals are obtained following a risk assessment by the Environmental Risk Management Authority (ERMA New Zealand). *Microctonus aethiopoides* is widely distributed in the Northern Hemisphere with different biotypes and strains, each adapted to a different set of hosts (Aeschlimann 1995).

Obtaining HSNO Act approval was facilitated considerably by discussion and advice from ERMA New Zealand, and information available on previous release applications for biological control agents. AgResearch applied for conditional rather than full release approval to allow restriction of the approval to the thelytokous parthenogenetic Irish strain only. In this way the potential adverse effects associated with hybridisation of other *M. aethiopoides* strains with the Moroccan biotype and loss of control of *S. discoideus* could be mitigated. AgResearch proposed, and ERMA New Zealand agreed to, a method to test new populations in containment to verify that individuals are thelytokous parthenogenetic.

Consultation with the Maori community was initiated as soon as confirmation was received that release required HSNO Act approval. Following advice from the Maori Unit at ERMA New Zealand, letters outlining the details of the proposal, and including postage-free response forms, were sent to 82 Iwi throughout New Zealand in December 2004. Subsequently, information folders (including a handout in Maori showing the life cycles of the parasitoid and weevil) were sent to the 13 Iwi that requested further details or expressed interest. AgResearch contracted Indigenous Corporate Solutions (IC Solutions) to facilitate and report on meetings with interested Iwi, which fostered useful open and friendly discussion.

As required under the HSNO Act the application was publicly notified. A total of 10 public submissions were received, six in support, three neutral or 'not opposed' and one from a private citizen opposing the release of the Irish parasitoid. Two of the submitters supporting the application presented their submissions at the public hearing on 12 October 2005. Following a risk assessment in which the benefits of releasing the Irish parthenogenetic strain were found to outweigh the risks and costs, the Authority approved the release, with controls for any future importations of the parasitoid.

The application was submitted on 23 May 2005 and approval was granted on 8 November 2005. The first release of the parasitoid was made on a farm near Morrinsville on 5 January 2006, with further releases in Hawke's Bay and Manawatu over the following six weeks. Establishment at the Hawke's Bay release site was confirmed by early April.

Ongoing research will monitor the buildup and spread of the parasitoid while native and introduced beneficial weevil species at the release sites will be sampled regularly to detect any parasitism of non-target species.

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