CHAPTER 9. *MICROCTONUS* PARASITOIDS AND NEW ZEALAND WEEVILS: COMPARING LABORATORY ESTIMATES OF HOST RANGES TO REALIZED HOST RANGES

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FORAGE WEEVIL PESTS IN NEW ZEALAND AND THEIR PARASITOIDS

The pest weevils *Sitona discoideus* Gyllenhal and *Listronotus bonariensis* (Kuschel) have been the targets of recent biological control programs in New Zealand forage crops. Two species of *Microctonus* parasitoids (Hymen.: Braconidae: Euphorinae) were introduced to control these pests. For these parasitoids, retrospective laboratory host range testing and field studies were carried out in order to test the degree to which laboratory data can predict field host ranges.

The Pest Weevils

Sitona discoideus in alfalfa Alfalfa is an important forage crop in low rainfall areas of New Zealand, particularly the eastern and central rain shadow areas of the South Island and parts of the North Island. Alfalfa out-produces grass pasture in the 300-800 mm rainfall zone (Douglas *et al.*, 1987). However, in the last 20 years, alfalfa production has declined, partly because of increased pressure from exotic pests (Douglas *et al.*, 1987). *Sitona discoideus* was first discovered in New Zealand in 1974 (Esson, 1975). Originating from the Mediterranean region, *S. discoideus* feeds on legumes in the genus *Medicago*. Adults feed on foliage, but the larvae are the most damaging stage, feeding within the root nodules of plants and reducing the ability of the plant to fix nitrogen. High densities of newly emerged adult *S. discoideus* can severely defoliate an alfalfa crop (Goldson *et al.*, 1984).

Listronotus bonariensis in pasture *Listronotus bonariensis* (Argentine stem weevil) was first recorded in New Zealand in 1927 (Marshall, 1937). The larva is a stem borer that feeds within the tillers of ryegrass and other grass species, and the adults feed on foliage. It has become one of New Zealand's most widespread and serious pests, thought to cause pasture production

losses of up to NZ$250 million per year. Losses occur as a result of reduced plant production, but also because the pest causes a change in pasture quality that can cause health problems in livestock (Prestidge *et al.*, 1991).

BIOLOGICAL CONTROL AGENTS

Microctonus aethiopoides For biological control of *S. discoideus*, the braconid parasitoid *Microctonus aethiopoides* Loan (Figure 1) was released in New Zealand in 1982 from Australia (Stufkens *et al.*, 1987), where it had been introduced earlier from the Mediterranean region (Cullen and Hopkins, 1982; Aeschlimann, 1983). Although ecotypes from Greece and France were introduced to Australia along with those from Morocco, it is thought that the Moroccan strain is the one that was released in New Zealand. Surveys throughout New Zealand have shown that *M. aethiopoides* is well established in *S. discoideus* populations in alfalfa-growing areas (Stufkens *et al.,* 1987; Ferguson *et al.*, 1994), where it has been shown to suppress *S. discoideus* populations (Goldson *et al.*, 1993). *Microctonus aethiopoides* was, however, released with limited host range testing in quarantine, which revealed no evidence of attack on non-target species (M. Stufkens, pers. comm.). In its natural range, *M. aethiopoides* is known to parasitize weevils in the genera *Hypera* (3 spp.) and *Sitona* (about 8 spp.) (Loan, 1975; Aeschlimann, 1980). A survey of alfalfa in southeastern Australia undertaken in November 2001 found evidence of non-target parasitism in only one Australian native species, an undescribed species of Prosayleus (Barratt *et al.*, in press).

Microctonus hyperodae This parasitoid (Figure 2) is of South American origin and was released in 1991 at several sites throughout New Zealand for control of *L. bonariensis*. This parasitoid has established successfully (Goldson *et al.*, 1994ab), although spread from the more southerly release sites has been slow (Ferguson *et al.*, 1997). *Microctonus hyperodae* was released after extensive quarantine testing (Goldson *et al.*, 1992). These tests suggested that the parasitoid was oligophagous, and the authors predicted that one or two native weevil species might be parasitized in the field. Collections of the parasitoid were made from seven South American locations from ecologically different environments in Argentina, Brazil, Uruguay, and Chile. These ecotypes were maintained separately in the laboratory rearing process, and for each major release, equal numbers of individuals of each of the ecotypes were released at each site so

Figure 1. *Microctonus aethiopoides* with *Sitona discoideus*. Photo: Mark McNeill. (UGA1295011)

Figure 2. *Microctonus hyperodae* with *Listronotus bonariensis*. Photo: Mark McNeill. (UGA1295012)

that information on differences in suitability of the different ecotypes could be determined. After three years it became apparent that the east coast ecotypes (Argentina, Brazil, Uruguay) had established more successfully than those from the west coast (Chile) (Goldson *et al.*, 1997).

Very little is known about the natural host range of *M. hyperodae*. Loan and Lloyd (1974) found that in the field in western Patagonia, *M. hyperodae* attacked only *L. bonariensis,* although other species in the genus were present. This apparent monophagy provided further evidence of the suitability of the parasitoid for biological control of *L. bonariensis* in New Zealand.

IDENTIFYING POTENTIAL NON-TARGET HOSTS IN NEW ZEALAND

Affinities between the Target Pests and the New Zealand Fauna

Likely potential non-target hosts of *M. aethiopoides* and *M. hyperodae* were native New Zealand weevils found near agricultural areas where the biological control agents were released. The target hosts for *M. aethiopoides* (*S. discoideus*) and *M. hyperodae* (*L. bonariensis*) are in the subfamily Entiminae, tribes Sitonini and Rhytirhinini, respectively, using the classification scheme of Leschen *et al*. (2003). Alonso-Zarazaga and Lyal (1999) considered the Sitonini and Tropiphorini to be so closely related that they should perhaps be combined. Tropiphorini and Rhytirhinini are well represented in New Zealand by native species, especially the former, many of which inhabit pastures and natural grasslands (Table 1). The native weevils of New Zealand are not well known taxonomically, and many species in these tribes are undescribed, with some probably still undiscovered. Knowledge of the ecology and biology of these native weevils is limited, which makes it difficult to know if the phenology of susceptible stages of the native species resembles that of the introduced pest weevils. Conversely, if native weevils are present at times when the target hosts are scarce, such timing might place the native species at increased risk. Information for some species has been gathered (Barratt *et al.*, 2000).

Table 1. Numbers of native weevils potentially at risk from *Microctonus* spp. introductions in New Zealand.

	Number of native weevil species with given degree of relatedness to introduced weevil (*Sd* or *Lb*) or number in group of special concern	
	S. discoideus	*L. bonariensis*
In same genus	0	0
In same tribe	23[1]	4
In same subfamily (Entiminae)	31	31
Valued biological control agents	2	2
Species of conservation concern	19	19

[1]includes Sitonini + Tropiphorini

To determine which New Zealand weevils might be at risk from these introduced biological control agents, a survey of over 150 pastures and alfalfa fields was carried out as part of a retrospective case study. Eighty-five species of Curculionoidea were collected, of which 75% were native (Barratt *et al.*, 1998). Thirty-two species were in the Tropiphorini, of which 84% were in the endemic genera *Irenimus* and *Nicaeana*. At many sites, species of native weevils (especially entimines) and the non-native pests were found in mixed populations at similar population densities (Barratt *et al.*, 1998). Furthermore, both of the exotic pest weevils were frequently found up to sub-alpine elevations in native vegetation (Dickinson *et al.*, 1998). Consequently, many additional native weevil species in a wide range of agricultural and natural grassland environments could potentially come into contact with the biological control agents. In addition, three native species of *Microctonus* parasitoids were discovered in New Zealand (Shaw, 1993), which are potentially at risk of being displaced by the introduced parasitoids. For only one of these species, *Microctonus zealandicus* Shaw (a gregarious parasitoid), is the host known: the native entimine *Irenimus aequalis* Broun.

AT RISK SPECIES OF SPECIAL VALUE

Two weevils, *Rhinocyllus conicus* Froelich and *Trichosirocalus horridus* (Panzer), have been introduced into New Zealand to control the target weed nodding thistle (*Carduus nutans* L.) in pasture and alfalfa. These two weed biological agents are likely to come into contact in the field with the parasitoids *M. aethiopoides* and *M. hyperodae* and might be parasitized.

The New Zealand Department of Conservation has in recent years recognized invertebrates as an important and dominant component of New Zealand's indigenous biodiversity. Among the invertebrates listed in recent reports as either 'nationally critical' (with a high risk of extinction) or 'nationally endangered' are four weevils (Hitchmough, 2002). Fifteen other weevils are considered 'nationally threatened', requiring conservation action (McGuinness, 2001).

DEVELOPMENT OF SPECIES LIST FOR HOST RANGE TESTING

Host range testing for *M. aethiopoides* was carried out in the early 1980s, when regulatory requirements for demonstrating environmental safety were less rigorous, and the methodologies used were not published or well documented. For *M. hyperodae*, host range testing was much more thorough and the results were well documented (Goldson *et al.*, 1990). These prerelease laboratory tests showed that four out of 23 test species were parasitized by *M. hyperodae*, and in all but one species, parasitoid development was unsuccessful or retarded (Goldson *et al.*, 1992). For both biological control agents, further testing was conducted as part of a retrospective study to determine the extent to which laboratory host range testing might have predicted the field host range (Barratt *et al.*, 1997). The rationale for selecting a list of non-target species for testing is shown in Table 2, which also lists some species tested subsequently.

Since our knowledge of the native weevil fauna is far from complete in New Zealand, it is not possible to calculate the proportion of the species of Entiminae that were represented in laboratory tests. Only four of a total of 31 genera found on the two main islands were included in tests, but the proportion of species tested would be less than 5%.

Table 2: Species selected (✓) for retrospective host range testing with *Microctonus aethiopoides* (*Ma*) or *Microctonus hyperodae* (*Mh*) and rationale for choosing each species.

Species	*Ma*	*Mh*	Rationale
I. Same as Target at Order Level[a]			
Allocharis sp. (Chrysomelidae)	✓		
II. Same as Target at Family Level[b]			
Peristoreus cruciger (Broun)	✓		Native Curculioninae, common in native grasslands
Rhinoncus australis Oke	✓	✓	Introduced Curculioninae, common in North Island pastures
III. Same as Target at Subfamily Level[c]			
Phlyctinus callosus Boheman	✓		Introduced pest species, same subfamily as target, Entiminae
IV. For *Ma* same as Target at Tribe Level[d]			
Irenimus aemulator (Broun)	✓	✓	
Irenimus aequalis (Broun)	✓	✓	
Irenimus egens (Broun)	✓	✓	
Irenimus stolidus Broun	✓	✓	
Irenimus similis (Barratt & Kuschel)	✓		
Nicaeana cervina Broun	✓	✓	
Nonnotus albicans Broun	✓		
Protolobus porculus (Pascoe)	✓		Very limited distribution in native and agricultural grassland
Zenagraphus metallescens Broun	✓		Native, common in South Island sub-alpine grasslands
V. For *Mh* same as Target at Tribe Level[d]			
Steriphus delaiguei (Germain)	✓	✓	Introduced; common in agricultural grassland
Steriphus variabilis Broun	✓		Native; common in native and agricultural grassland
VI. Same as Target at Genus Level[e]			
Sitona lepidus Gyllenhal	✓	✓	Introduced pest

Table 2: Species selected (✓) for retrospective host range testing with *Microctonus aethiopoides* (*Ma*) or *Microctonus hyperodae* (*Mh*) and rationale for choosing each species (continued).

Species	*Ma*	*Mh*	Rationale
VII. Species in same genus as endangered species			
Anagotus latirostris (Broun)		✓	Native, limited distribution in Central Otago alpine herbfield
VIII. Biological control agents			
Rhinocyllus conicus Froelich	✓	✓	Introduced weed biological control agent
Trichosirocalus horridus (Panzer)	✓	✓	Introduced weed biological control agent

[a]Beetles other than weevils (Curculionidae); species found in native grassland that have similar behavioral and habitat characteristics and similar size to one of the target hosts.
[b]In the Curculionidae, but in subfamilies different from that of the target pests, i.e., not in Entiminae
[c]In the Entiminae, but not in either of the tribes Triopiphorini or Rhytirhinini
[d]For M. aethiopoides this is other species of Tropiphorini; for M. hyperodae, this is Rhytirhinini; this group is composed of species considered at risk because they inhabit native or agricultural grassland
[e]Ether another species of Sitona or Listronotus

LABORATORY HOST RANGE TESTS

Rearing Conditions

Tests were conducted in an insect rearing room maintained at 20 ± 2°C with a relative humidity of 40-60%, and a photoperiod of 16:8 (L:D) hours. This is within the range of 'average' field conditions that would be expected during the day in summer in New Zealand. Weevils were contained in plastic cages (160 by 180 mm by 75 mm deep) with a fine-gauze lid (Figure 3). The floor of the cage was fitted with plastic mesh with holes 1 by 1 mm, and this cage was inserted

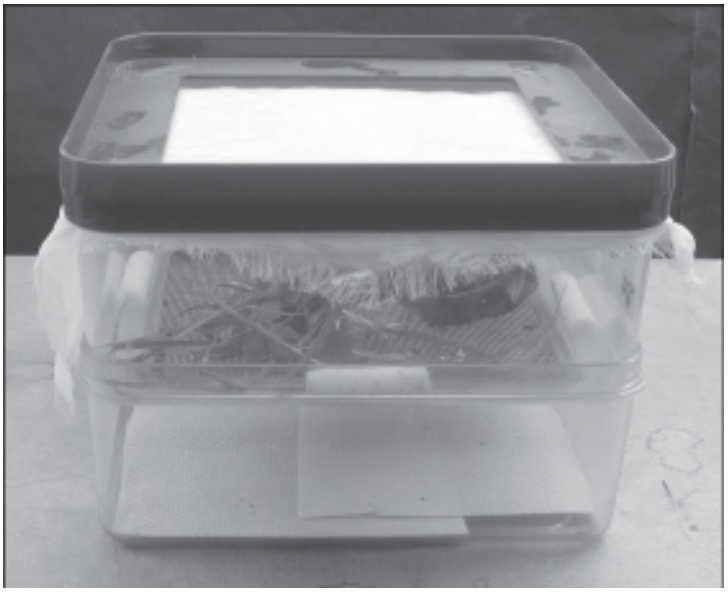

Figure 3. Cage used for standard host range tests. Photo: Barbara Barratt.

into the top of another similar container with textured absorbent paper towel covering the base. The paper served as a substrate for pupation of emergent prepupal parasitoids, which moved down through the mesh from the upper to the lower cage. Cages were selected on the basis of size. They needed to be large enough to spaciously accommodate about 20 weevils and two bundles of alfalfa, yet small enough to be handled easily, and housed in a controlled temperature rearing laboratory in large numbers to allow for experiments with adequate replication. In general, the aim of laboratory tests is to replicate 'natural' field conditions as closely as possible, but this is invariably a compromise. Host densities are likely to be much higher in cages than in the field (in our cages, weevil densities would be close to 700 per m^2, which could occur in the field at the upper range of density), and the environment within a cage is likely to be far less complex than the field environment. McNeill (2000) found that parasitoid activity and weevil parasitism was significantly higher in Petri dishes compared with laboratory cages, concluding that in a smaller space, the number of encounters between host and parasitoid increased. Evans *et al.* (1997) found that parasitism of native weevils by *M. aethiopoides* was 40-55% in laboratory cages as described above but averaged only 15% in large field cages (45 x 90 x 50 cm high). The level of parasitism obtained in the field cages was similar to that recorded in an open field population nearby. Proportions of failed parasitism and superparasitism in the field cages were similar to that in laboratory cages.

Insect Feeding

Listronotus bonariensis and *S. discoideus* were provided with Grasslands cv. Manawa ryegrass ([*Lolium perenne* x *L. multiflorum*] x *L. perenne*) and Grasslands cv. Wairau alfalfa, *Medicago sativa* L., respectively. Native weevils survived best in the laboratory when given both ryegrass and alfalfa, and pollen grains (Grainger, 1995). *Rhinocyllus conicus* was provided with foliage of nodding thistle. Ryegrass and alfalfa plants were grown in commercial seed-raising mix in glasshouse trays with cells 2.7 by 2.7 cm, 4.5 cm deep, sown with 6-10 seeds per cell. The plants were grown to »10 cm high, removed intact, and the roots and soil enclosed in a plastic bag (10 by 7.5 cm) secured firmly at the base of the plants using a plastic cable clip to prevent weevils entering the bags. One or two bags of plants were placed in each cage and replaced with fresh plants every 3 to 4 days. Water was supplied using saturated cotton dental wicks placed in the cages, which were resoaked every 1 to 2 days and at each change of food.

Test Species and Parasitoid Collection

Weevils were collected from the field, in most cases by using a commercial leaf-sucking machine (Blower Vac) fitted with a gauze collection bag immediately behind the inlet of the machine or by sweep-netting (Barratt *et al.*, 1997). The thistle biological control agents *R. conicus* and *T. horridus* were collected directly from thistle plants in the field, and the native weevils *A. latirostris* and *Zenagraphus metallescens* Broud were collected by hand from native vegetation.

Microctonus aethiopoides was reared from *S. discoideus* collected from the field, and *M. hyperodae* was reared from *L. bonariensis* from a laboratory colony at AgResearch, in Lincoln, New Zealand. Newly emerged parasitoids were provided with a water-honey solution and held for up to four days before being used in an experiment. *Microctonus aethiopoides* females were confined with males to allow mating to occur before being used in tests. This was not necessary for *M. hyperodae,* which is parthenogenetic.

Host Range Test Procedure

Using a no-choice design, a standard host/parasitoid ratio and procedure was followed as described in Barratt *et al.* (1996). Twenty weevils were placed in each cage (Figure 3) and exposed to three female parasitoids for 48 hours. Depending upon the availability of weevils, five replicate cages of the non-target test species were exposed and five cages were unexposed. Where possible, five exposed and five unexposed replicate cages of the target host were run in parallel with one or more of the test species to provide a positive control. After the 48-hour exposure period, parasitoids were removed and the weevils maintained until the resulting parasitoid prepupae emerged.

Emergent parasitoid pupae were recorded daily and removed from the cages to Petri dishes containing a water-soaked dental wick to maintain high humidity. Newly eclosed adults also were recorded daily, allowing comparison of parasitoid developmental periods between rearing from test and target species.

Each experiment was terminated when no further parasitoid prepupae emerged from weevils for at least two days, or after 30 days if no prepupae had emerged. Surviving weevils, as well as those that died during the experiment, were dissected (Figure 4). The presence of parasitoid larvae in these weevils was recorded and such hosts added to those which parasitoids had emerged to give total percentage parasitism. Any signs of a host immune response, including melanization of parasitoid eggs or larvae, encapsulation, or malformed, emaciated larvae were noted, as well as incidence of super-parasitism.

The testing procedure described above was standardized so that results would be comparable between tests, and a no-choice rather than choice design was used so that the maximum physiological host range could be observed. We were interested in determining the full range of

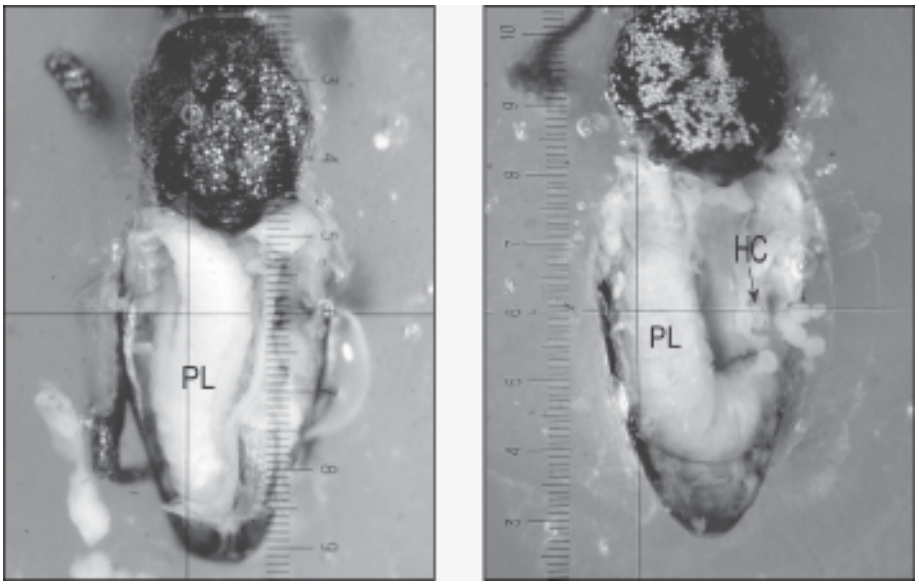

Figure 4. New Zealand native weevils dissected to show *M. aethiopoides* parasitoid larvae (PL) and 1st instar head capsule (HC). Photos: Barabara Barratt. (UGA1295013)

potential hosts rather than comparing host preferences. In the field, a choice of hosts may not always be available to parasitoids, especially if they emerge from hosts that have carried them away from the target host's environment. This seems to occur quite commonly when parasitized *S. discoideus* disperse after aestivation in summer. It is also quite common to find low numbers of *L. bonariensis* in native grassland, and parasitized *L. bonariensis* have been encountered in sub-alpine habitat. In the absence of the target hosts, as occurs when *S. discoideus* is in summer aestivation, parasitoids may be forced to seek suitable alternative hosts among native species.

INTERPRETATIONS OF TEST RESULTS

LABORATORY TEST RESULTS

Microctonus aethiopoides This species was been found to oviposit in 9 of 11 species of native Curculionidae (14 of 19 total Curculionidae species) to which it was exposed in the laboratory (Table 3), with levels of parasitism often equivalent and sometimes higher than those achieved in the target host, *S. discoideus,* in parallel tests. Immature parasitoid development times in native weevils (from when adult parasitoids were removed from test cages to when parasitoid prepupae emerged from the test species) were similar to that in *S. discoideus*. Of parasitoids that emerged as pre-pupae from native species, about 80% developed to the adult stage, but 34% of the parasitoids found during dissection (of hosts from which no parasitoids emerged) were melanized or showed other signs of a host immune response. Most of these cases occurred in the test species that were most distantly related to the target host. No melanized larvae were found in dissections of *S. discoideus.*

In a later laboratory study, Barratt and Johnstone (2001) found that superparasitism occurred in the native host *Nicaeana cervina* Broun more frequently than would be expected. We also found that successful development of *M. aethiopoides* larvae in *N. cervina* was more likely to occur if the host had been superparasitized, suggesting that multiple parasitism helps suppress host defences in this novel host. A virus-like particle (MaVLP), structurally similar to polydnavirus, has been found in the ovaries of female *M. aethiopoides* (Barratt *et al.*, 1999), and this or other parasitoid-derived secretions may be transmitted to hosts during parasitoid oviposition, as is the case in other braconids and ichneumonids (Beckage, 1998).

Both of the two weed biological control agents tested, *R. conicus* and *T. horridus*, were parasitized in laboratory tests, although in the case of *T. horridus,* this was recorded only once and the parasitoid larvae did not develop successfully. About 40% of *R. conicus* exposed to *M. aethiopoides* were parasitized successfully, and parasitism of this host has also been recorded in the field (Table 3). Although *R. conicus* was exposed to *M. aethiopoides* in pre-release tests, no parasitism was recorded at that time; this may have been because the tests were undertaken in autumn when *R. conicus* was probably in diapause and inactive. It is known that *M. aethiopoides* requires an active host to stimulate stalking behaviour and oviposition (Loan and Holdaway, 1961).

Table 3. Results of laboratory testing (L) with *M. aethiopoides* (*Ma*) and *M. hyperodae* (*Mh*) and parasitism recorded in the field (F) either by rearing or dissection.

Test species	*Ma*		*Mh*		Habitat and Reference
	L	F	L	F	
Curculionoidea					
Anagotus latirostris (Broun)	-	-	N	-	alpine cushionfield; Goldson *et al.*, 1992 for Mh in lab; Barratt *et al.*, 1997
Atrichonatus taeniatulus (Berg)*	-	Y	-	N	alfalfa; Barratt *et al.,* 1997
Brachyolus obscurus Sharp	-	N	-	N	pasture
Bryocatus spp.	-	N	-	N	native grassland and developed pasture
Catoptes censorius Pascoe	-	N	-	N	pasture
Catoptes cuspidatus (Broun)	-	N	N	N	native grassland/shrubland; Goldson *et al.*, 1992 for *Mh* in lab
Catoptes murinus (Broun)	-	N	-	N	native sub-alpine hebfield
Catoptes robustus Sharp	-	N	Y	N	alpine cushionfield; Goldson *et al.*, 1992 for *Mh* in lab
Catoptes sp. cf. *scutellaris* Sharp	-	N	-	N	shrubland
Cryptorhynchinae sp.	-	N	-	N	native grassland
Eugnomus sp.	-	Y	N	N	subalpine herbfield; Goldson *et al.*,1992 for *Mh* in lab
Desiantha sp.	-	-	N	-	pasture; Goldson *et al.*, 1992 for *Mh* in lab
Epitemetes grisealis Broun	-	N	-	N	Pasture
Epitimetes sp.1	-	N	-	N	Pasture
Epitimetes sp.2	-	N	-	N	Pasture
Exapion ulicis (F.)	-	N	N	N	introduced gorse biocontrol agent; Goldson et al., 1992 for Mh in lab
Gromilus sp.	-	-	N	-	forest; Goldson *et al.*, 1992 for *Mh* in lab
Hoplocneme cyanea	-	-	N	-	forest; Goldson *et al.*, 1992 for *Mh* in lab

Y = parasitism recorded in test or sample; N = parasitism not recorded; - = species not tested; * = introduced species.

Table 3. Results of laboratory testing (L) with *M. aethiopoides* (*Ma*) and *M. hyperodae* (*Mh*) and parasitism recorded in the field (F) either by rearing or dissection (continued).

Test species	*Ma*		*Mh*		Habitat and Reference
	L	F	L	F	
Curculionoidea (continued)					
Irenimus aemulator (Broun)	Y	Y	Y	N	native grassland and pasture; Barratt *et al.*, 1997
Irenimus aequalis (Broun)	Y	Y	Y	Y	pasture; Barratt et al.,1997; Goldson *et al.*, 1992 for *M*h in lab
Irenimus albosparsus (Broun)	-	Y	-	N	pasture; Barratt *et al.*, 1997
Irenimus compressus (Broun)	-	N	N	N	pasture; Goldson *et al.*,1992 for *Mh* in lab
Irenimus egens (Broun)	Y	Y	Y	N	pasture; Barratt *et al.*, 1997
Otiorhynchus sulcatus (F.)	N	N	-	N	garden
Peristoreus cruciger (Broun)	N	N	-	N	olive trees
Peristoreus veronicae (Broun)	-	-	N	-	Goldson *et al.*, 1992 for *Mh* in lab
Peristoreus sp.	-	N	-	N	native grassland/shrubland
Praolepra infusca Broun	-	N	N	N	native shrubland; Goldson *et al.*,1992 for *Mh* in lab
Protolobus porculus Pascoe	Y	N	N	N	pasture; Barratt *et al.*, 1997
Phlyctinus callosus Boheman	N	N	N	N	pasture; Barratt *et al.*,1997
Rhadinosomus acuminatus	-	-	N	-	Goldson *et al.*,1992 for *Mh* in lab
Rhinocyllus conicus (Froelich)*	Y	Y	N	N	introduced thistle biological control agent; Barratt *et al.*, 1997 and Goldson *et al.*,1992 for *Mh* in lab
Rhinoncus australis Oke*	Y	N	N	?	Barratt *et al.*,1997; positive *Mh* in the field unconfirmed
Rhopalomerus sp.	-	-	N	-	forest; Goldson *et al.*, 1992 for *Mh* in lab
Sitona discoideus Gyllenhal	Y	Y	N	N	alfalfa
Sitona lepidus Gyllenhal*	Y	Y	N	Y	pasture; Barratt *et al.*, 1997

Y = parasitism recorded in test or sample; N = parasitism not recorded; - = species not tested; * = introduced species.

Table 3. Results of laboratory testing (L) with *M. aethiopoides* (*Ma*) and *M. hyperodae* (*Mh*) and parasitism recorded in the field (F) either by rearing or dissection (continued).

Test species	*Ma*		*Mh*		Habitat and Reference
	L	F	L	F	
Curculionoidea (continued)					
Steriphus ascitus (Pascoe)	-	-	N	-	pasture; Goldson *et al.*,1992 for *Mh* in lab
Steriphus diversipes lineatus (Pascoe)	-	N	N	N	pasture; Goldson *et al.*, 1992 for *Mh* in lab
Steriphus variabilis Broun	Y	Y	N	Y	pasture; Goldson *et al.*, 1992 for *Mh* in lab; Barratt *et al.*, 2000 for *Mh* in field
*Trichosirocalus horridus Panzer**	Y	N	N	N	introduced thistle biocontrol agent; Goldson *et al.*, 1992 for *Mh* in lab; Barratt *et al.*, 1997
Zenagraphus metallescens Broun	Y	N	-	N	alpine herbfield; Barratt *et al.*, 1997
Total non-target positives	14	16	7	3	
Total negatives	5	33	24	45	
Grand total (non-target)	19	49	31	48	
% positives (non-target)	73.7	32.6	22.6	6.3	
Other Coleoptera					
Eucoides suturalis Pascoe (Cerambycidae)		N		N	Pasture
Chaetocnema nitida (Broun) (Chrysomelidae)		N		N	native grassland
Allocharis sp. (Chrysomelidae)	N	N		N	sub-alpine herbfield
Archeocrypticus topali Kaszab (Archeocrypticidae)			N		alfalfa

Y = parasitism recorded in test or sample; N = parasitism not recorded; - = species not tested; * = introduced species.

In tests with *M. aethiopoides*, dissections showed that 2% of the field-collected *I. aemulator* weevils used in tests as controls (not exposed to parasitoids) had already been parasitized in the field by *M. aethiopoides.*

Microctonus hyperodae Combining pre-release studies (Goldson *et al.*, 1992) and retrospective host range tests on *M. hyperodae* (Barratt *et al.*, 1997), 31 weevil species were exposed to this parasitoid, of which 21 were New Zealand native species, and successful oviposition occurred in five species. However, non-target parasitism levels were much lower in comparison with parallel tests with *L. bonariensis*. Furthermore, in the five native species parasitized, para-

sitoids emerged from only 3% of the weevils exposed. The proportion of parasitoids found during dissection that were melanized or showed other signs of a host immune response was over 40% in native weevils, compared with about 8% in *L. bonariensis*. Unwanted parasitism in field-collected weevils used in tests was low (3% of the unexposed *N. cervina* and 4% of the unexposed *I. aemulator* were parasitized by *M. aethiopoides* from earlier field parasitism).

The "endangered" weevils now listed in New Zealand as of conservation concern are in the genera *Lyperobius*, *Anagotus*, *Stephanorhynchus,* and *Hadramphus*. The work reported here predates this designation. One species of *Anagotus* (*A. latirostris*) was included in the list of weevils exposed to *M. hyperodae* both before its release (Goldson *et al.*, 1992) and in retrospective host range tests (Barratt *et al.*, 1997), and in both cases results were negative (Table 3).

FIELD PARASITISM

Eleven New Zealand native species and five introduced species have been found to be parasitized by *M. aethiopoides* in the field (Table 3), mostly in the agricultural environment. However, *L. bonariensis* collected in modified vegetation (a ski field) at 1650 m were found parasitized by *M. aethiopoides*, and a number of other native weevils collected from native grassland at 500 to 1000 m have also been found parasitized by *M. aethiopoides*. Excluding records where the sample size was less than 10, parasitism levels ranged from 1.6 to 71.4%, the highest parasitism being in *I. aemulator* collected from a pasture in Otago. Of the eleven weevil species parasitized by *M. aethiopoides* in the laboratory, three have as yet not been found parasitized in the field – *P. porculus*, *Z. metallescens*, and *T. horridus*.

Microctonus hyperodae has been recovered from three non-target species in the field: the native species *I. aequalis* and *Steriphus variabilis* Broun, and the recently discovered exotic species *Sitona lepidus* Gyllenhal (Barratt *et al.,* 1996). Both *I. aequalis* and *S. lepidus* host records were from Waikato (North Island of New Zealand), and in both instances, only a single parasitized host was found. A small number of *S. variabilis* have been found parasitized by *M. hyperodae* in the South Island in Canterbury (Barratt *et al.*, 2000) in pasture where parasitism of the target host was moderately high at the time when non-target parasitism occurred.

COMPARISON BETWEEN PREDICTED AND REALIZED FIELD PARASITISM

Predictions about the likely host range of *M. aethiopoides* and *M. hyperodae,* which were made after retrospective laboratory investigations were complete and for *M. hyperodae* before its release, were generally borne out by what was found in field studies. *Microctonus aethiopoides* has proved to be polyphagous, developing successfully with quite high levels of parasitism in a variety of non-target taxa in a range of habitats. *Microctonus hyperodae* has to date proved perhaps even more oligophagous than was anticipated with only three confirmed nontarget species, and indeed very few individuals having been discovered parasitized in the field. Significantly, one of those detected in the field was the native species *I. aequalis,* which was predicted from the quarantine investigation to be a likely host for *M. hyperodae* (Goldson *et al*., 1992).

In this comparison between the two parasitoid species, allowance must be made for the fact that *M. hyperodae* is currently less widely distributed throughout New Zealand than *M. aethiopoides* and has been present for only 13 years, compared to 22 years for *M. aethiopoides.*

In general, weevils in the Entiminae appear to be more at risk from parasitism by *M. aethiopoides* than more distantly related taxa, as might be expected. This manifests itself in terms of a higher proportion of weevils attacked in the laboratory and more successful parasitoid larval development. However, field studies have shown that coexistence in the same habitat is also an important factor in susceptibility to attack by parasitoids. For example, *R. conicus* (Curculioninae), which is not uncommonly parasitized by *M. aethiopoides* in the field, is more distantly related to *S. discoideus* than, say, the genus *Catoptes* (Entiminae), which has not been recorded in the field as a host for *M. aethiopoides*. However, *R. conicus* is often found on nodding thistle (*C. nutans*) plants growing as weeds in alfalfa, and hence comes into contact with *M. aethiopoides*. In contrast, many *Catoptes* species are found in shrubland, a habitat where *M. aethiopoides* is likely to be less common.

Unfortunately the higher classification of Curculionoidea remains a contentious issue, and so it is not possible to analyse phylogenetic relationships between taxa and determine whether phylogeny and potential host range are closely linked, as tends to be the case for weed biological control agents and their host plants. As indicated above, while 'relatedness' might determine physiological host range at a broad level, ecological affinity and insect behaviour also appear to be important determinants of field non-target parasitism in this system.

PROBLEMS ENCOUNTERED

OBTAINING TEST SPECIES

Collecting sufficient specimens from the field for host range tests was sometimes difficult, especially from natural as opposed to agricultural grassland areas. In some cases, this was a limitation when attempting to design robust, well replicated tests. Rare and endangered species could not be included in tests, even though these are the very species of greatest concern. In these instances, however, other species in the same genus were tested, when possible.

INSECT PHYSIOLOGICAL CONDITION

It is important to ensure that the individuals of a test species used in a host range test are in an appropriate physiological condition when presented to parasitoids. *Microctonus* species require an active host so that the wasps are stimulated to approach and stalk a potential host. If the host is either moribund or in a physiologically quiescent state (e.g., in diapause), the wasp will not attempt to oviposit and hence will give a misleading result. We consider that this may have occurred when host range testing was being carried out with *M. aethiopoides* before its release. At that time, *R. conicus* was included in tests but no parasitism was recorded. Subsequent tests have shown that in fact *M. aethiopoides* does parasitize *R. conicus* in the laboratory and in the field. We believe that the original tests were carried out in autumn when *R. conicus* is normally in diapause and very inactive. It is possible that approval to release *M. aethiopoides* into the field may not have been granted had it been shown that *R. conicus*, a weed biological control agent, might be adversely affected.

The plants present during a test should be standardized so that test and control insects are held in similar circumstances to avoid any differential effects occurring as a result of plant-derived volatiles. For example, if an alfalfa-feeding insect is being exposed to a parasitoid in parallel with the target host which is a grass-feeder, then both the appropriate grass species and alfalfa should be placed in all cages.

SUMMARY

A retrospective study of non-target parasitism by *Microctonus* spp. in New Zealand was carried out with the objective of developing widely applicable, robust, but feasible methods for pre-release evaluation of non-target effects of proposed biological control agents. The research aimed to contribute to improved decision support for the appropriate regulatory agency in New Zealand, the Environmental Risk Management Authority (ERMA New Zealand), and the Department of Conservation. The study compared laboratory tests, which could be carried out in quarantine, to the host ranges as realized in the field for validation.

Since no biological control agent safety testing program can be totally exhaustive, there is a sequence of steps that can be taken to minimize the chances of adverse effects. Largely, these are based upon protocols adopted by weed biological control practitioners and adapted to suit insect parasitoids. Lack of complete taxonomic and ecological information about the insect fauna in most countries (e.g., compared with plants) makes prediction difficult.

The case studies reported here have hopefully provided some useful information that can be adopted for pre-release host range testing of any parasitoid. To summarize, we have found that the following points may be particularly important:

- Understand as fully as possible the phylogeny, ecology, and phenology of the target host(s) of the proposed biological control agent.
- Identify as fully as possible the elements of the fauna in the area of proposed introduction that might be at risk as a result of taxonomic and ecological affinity to the target pest.
- Consider parasitoid ecotype (or geographic origin) if comparisons are being made with other biological control programs using the same parasitoid species.
- Consider testing species of special economic or conservation interest (or their congeners).
- Conduct well replicated host ranges tests with controls under conditions that optimize the physiological condition of both test species and parasitoids, and provide standardized test conditions.
- Standardize host-parasitoid ratios and exposure times, choosing conditions that give high levels of parasitism in the target host as a basis for comparison with test species.
- Use no-choice tests initially for a conservative test; choice tests can contribute different information but probably are less informative than for weed biological control agents.

- When dissecting hosts to record parasitism, make detailed records of the reproductive status/fecundity of hosts, the incidence of superparasitism, and any evidence of a host immune response (such as melanization or abnormal development of parasitoid immature stages).
- Test laboratory predictions of non-target parasitism in post-release field studies.

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