COLLECTIONS OF *MICROCTONUS AETHIOPOIDES* LOAN (HYMENOPTERA: BRACONIDAE) FROM IRELAND

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ABSTRACT

The biological control programme against *Sitona lepidus* (Gyllenhal) (Coleoptera: Braconidae) commenced in 1998, with the first parasitised weevils imported into New Zealand quarantine in 2000. Extensive collections in Europe confirmed that the solitary endoparasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) was the principal natural enemy of adult *S. lepidus*. With one exception, all *M. aethiopoides* reared from *S. lepidus* collected in Europe have been arrhenotokous. All *M. aethiopoides* collected from Ireland are thelytokous, which obviates the risk of hybridisation with an arrhenotokous Moroccan strain already established in New Zealand. Levels of parasitism in *S. lepidus* field-collected from Ireland were low (<8%) and overall averaged 0.7%. Rates of parasitism of *S. lepidus* in New Zealand quarantine have averaged 25%, but ranged from 0 to 95%. Aspects relating to the rearing and management of Irish *M. aethiopoides* are discussed including possible reasons for low rates of parasitism in quarantine.

Keywords: clover root weevil, *Sitona lepidus*, classical biological control, parasitoid strains, biotypes.

INTRODUCTION

Since its discovery in New Zealand in 1996 (Barratt et al. 1996), the white clover (*Trifolium repens*) L. pest *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), has been the subject of research investigating the potential of classical biological control through the introduction of a suitable natural enemy (Goldson et al. 2001, 2004). Of European origin (Campbell et al. 1989), *S. lepidus* is distributed widely throughout Europe to Siberia, Asia Minor and North America (Hoffman 1950; Bright 1994). Based on previous research on the genus *Sitona* in Europe and North Africa, several natural enemies of the adult weevil are known (Aeschlimann 1980, 1983). Surveys in the USA and Europe to assess the natural enemy complex attacking *S. lepidus* found that the most promising candidate was the parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) (Goldson et al. 2001). A solitary endoparasitoid of the adult weevil, biotypes of *M. aethiopoides* are an important biological control agent of *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) in the USA (Kingsley et al. 1993) and *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) in New Zealand (Goldson et al. 1990b; Barlow & Goldson 1993).

In 2001 and again in 2002, collections of *S. lepidus* were made from several countries in the European Union. The weevils were then forwarded by air cargo into quarantine at AgResearch Lincoln, New Zealand (Goldson et al. 2001). Colonies of *M. aethiopoides* from several countries in Europe were established for ongoing research into parasitism and host specificity studies. An unexpected discovery was that *M. aethiopoides* from

Ireland was thelytokous whereas all other populations derived from Romania, France, Netherlands, Scotland, Wales, England and Finland were arrhenotokous. This was important, because inter-ecotype mating between the other European strains with the Moroccan biotype already released in New Zealand against *S. discoideus* Gyllenhal, produced hybrids that were less effective against their respective hosts (Goldson et al. 2003). The Moroccan biotype does not successfully parasitise *S. lepidus*. Therefore, the Irish parasitoid was selected as a potential candidate for introduction to New Zealand because there is negligible risk of inter-ecotype mating occurring between the thelytokous Irish and arrhenotokous Moroccan *M. aethiopoides* and it was relatively host specific (Gerard et al. 2005). This paper reports on aspects of the collection of *M. aethiopoides* from Ireland and subsequent quarantine findings.

MATERIAL AND METHODS

Collections from Ireland

The parasitoid lines from Ireland established in quarantine originated from *S. lepidus* collected from *Trifolium repens* L./*Lolium perenne* L. pasture in Ireland. The majority of these collections were made by AgResearch staff, and because of logistical constraints were of limited duration in autumn and spring. Because *T. repens* is not widely utilised as a species in pastoral farming in Ireland (Humphries et al. 2005), collection sites were restricted to research farms run by Teagasc in Ireland and the Department of Agriculture and Rural Development (DARD) in Northern Ireland. Collections were made from pasture near Leenuan (Co. Galway), Athenry (Co. Galway), Solohead (Co. Tipperary), Moorepark (Co. Cork), Johnstown Castle (Co. Wexford), Oakpark (Co. Carlow), The Grange (Co. Meath) and Belfast (Co. Down) (Fig. 1). *Sitona lepidus* were collected with a motorised blower-vac, which was used to suck the insects into a removable net recessed into the inlet pipe. The litter was sieved and weevils were collected by hand. Weevils were transferred into 35 ml vented containers furnished with cardboard strips and *T. repens* leaves for food. Each container fitted into a polystyrene case, which was sealed, and in turn sealed inside a cardboard box. The boxes were labelled and then forwarded by air cargo to New Zealand. Following clearance from border inspection officials of the Ministry of Agriculture and Forestry, the boxes were opened in an insect quarantine facility at AgResearch Lincoln.

Quarantine-based parasitism

To establish the quarantine colonies, all parasitoid adults were exposed individually to 30–40 New Zealand *S. lepidus* for 48 to 72 h in a vented clear polycarbonate cage (220 mm × 130 mm × 75 mm depth). Two to three bouquets of glasshouse grown *T. repens* (cv. Kopu II) were included as food for the weevils. The parasitoid was removed at the end of the exposure period and the weevils were transferred to a parasitoid rearing cage consisting of an upper and lower chamber (McNeill et al. 2002). Prepupae emerging from weevils held in the upper chamber were collected in the lower chamber where they pupated. Once they were at least 48 h old, cocoons were removed and placed in a Petri dish. A dental roll moistened with water prevented desiccation of the developing parasitoid adult.

Because *S. lepidus* had not been found in the South Island until 2006, maintenance of the parasitoid colonies was dependent on weevils collected every 7–20 days from *T. repens*/*L perenne* pastures near Ruakura. Parasitoid-exposed weevils were fed twice weekly, with weevil mortality and parasitoid prepupal emergence recorded weekly. Total parasitism was recorded when all of the parasitoids had ceased to emerge from a line.

RESULTS AND DISCUSSION

Field parasitism

Between 12 July 2000 and 01 April 2004, five collection trips were made with a total of 11,257 *S. lepidus* collected from nine locations in Ireland and Northern Ireland (Fig. 1).

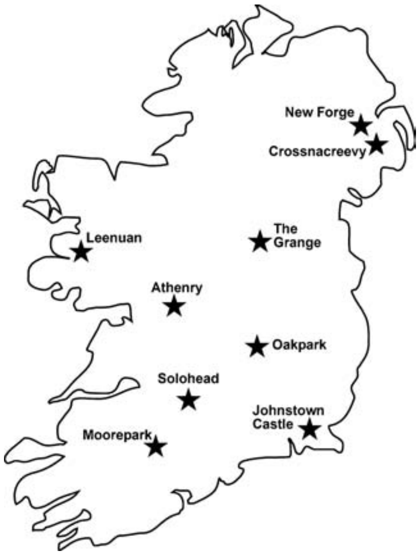

FIGURE 1: *Sitona lepidus* collection sites in Ireland 2000–2004.

In 2000, initial screening was done in a temporary laboratory in England (Goldson et al. 2001) and ca 1200 *S. lepidus* collected from Athenry were sent to England, from which three parasitoids were recovered (Table 1). Thereafter, 10,055 *S. lepidus* were sent by airfreight from Ireland to New Zealand. At Lincoln, the weevils were held in parasitoid rearing containers to allow any immature parasitoids to develop and emerge from the weevils. Adult *M. aethiopoides* were identified using Loan (1975).

Survival of weevils in transit was very good, averaging 98.5%. In general, levels of parasitism in *S. lepidus* collected from these locations were very low (Table 1), with a maximum level of parasitism of ca 7% found in weevils collected from a paddock near Athenry in September 2001 (Table 1). A total of 79 pupae were recovered, from which 52 female *M. aethiopoides* emerged. Neither *M. aethiopoides* males nor any other parasitoid species were recovered. Several collections failed to yield any parasitoids, despite timing visits to coincide with successful collections made from Athenry in September 2001 (Table 1). None of the weevils that died in quarantine were dissected to determine if they had been parasitised and it is conceivable that premature mortality of parasitised weevils led to the actual parasitism level being significantly under-estimated. For example, parasitism of field collected Argentine stem weevil *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) by *Microctonus hyperodae* Loan (Hymenoptera: Braconidae), as indicated by dissection of a weevil subsample, has at times been significantly higher when compared to prepupal emergence (M.R. McNeill, unpubl. data). This result is attributed to premature mortality of the parasitised weevil proportion of the caged population. Alternatively, collections may have missed the peak level of parasitism of Irish *S. lepidus*. In the classical biological control programme

TABLE 1: Locations, collection dates and number of *S. lepidus* collected in Ireland, with associated rates of parasitism.

Location, County	Collection date	Site	No. *S. lepidus* collected	No. *M. aethiopoides* pupae	% parasitism	No. adult parasitoids
Athenry, Galway	12 Jul 00	1	56	0	0	
Athenry, Galway	3-Aug 00	1	126	0	0	
Athenry, Galway	20 Sep 00	1	850	5	0.6	3
Athenry, Galway	19 Oct 00	1	170	1	0.6	1
Athenry, Galway	19-21 Sept 01	1	637	16	2.5	15
Athenry, Galway	19-21 Sept 01	2	1147	20	1.7	10
Athenry, Galway	19-21 Sept 01	3	270	19	7.4	13
Athenry, Galway	1-2 Oct 02	1	62	0	0	
Athenry, Galway	1-2 Oct 02	2	146	0	0	
Athenry, Galway	1-2 Oct 02	3	165	0	0	
Athenry, Galway	1-2 Oct 02	4	141	0	0	
Athenry, Galway	9-12 Sep 03	1	3418	0	0	
Leenane, Galway	19-21 Sept 01	4	33	0	0	
Moorepark, Cork	4 Oct 02	1	29	0	0	
Solohead, Tipperary	31 Mar 04	1	443	3	0.7	1
Oakpark, Carlow	18-19 Sep 03	1	236	1	0.4	
Oakpark, Carlow	30 Mar 04	1	577	9	1.6	6
The Grange, Meath	22 Sep 03	1	392	0	0	
The Grange, Meath	22 Sep 03	2	158	0	0	
The Grange, Meath	29 Mar 04	1	204	1	0.5	
Johnstown Castle, Wexford	19 Sep 03	1	142	0	0	
Johnstown Castle, Wexford	31 Mar 04	1	400	0	0	
Crossnacreavey, Down	17 Sep 03	1	307	2	0.7	2
Loughgall Field, Down	16 Sep 03	1	385	1	0.3	
Loughgall Field, Down	16 Sep 03	2	294	0	0	
Newforge Lane, Down	16 Sep 03	1	277	1	0.4	1
Newforge Lane, Down	1 Apr 04	1	192	0	0	

targeting *L. bonariensis*, low levels of parasitism (0.7–5.4%) were recorded for *M. hyperodae* reared in New Zealand quarantine from *L. bonariensis* collected in South America (Goldson et al. 1990a), although higher rates of parasitism were recorded in earlier surveys (Loan & Lloyd 1974; Goldson et al. 1990a). Despite this, the parasitoid is an effective biological control agent in the New Zealand agricultural environment where it has been shown to effect significant reductions in adult *L. bonariensis* populations (Goldson et al. 1998).

Quarantine-based parasitism

Not all prepupae that emerged from Irish *S. lepidus* successfully pupated nor was there 100% survival from the pupal to adult stage. Adult parasitoids were collected within 48 h of emergence and placed in a 5 ml screw-cap clear plastic vial and supplied with 20% honey water solution presented on a small section of filter paper. They were then held at 8°C and 14:10 h light:dark until required for continuation of the colonies or use in other studies (Goldson et al. 2005; McNeill et al. 2005). Individual parasitoids have been maintained as separate lines, and are still being reared in 2006. Depending on the year of collection, these lines have completed between 16 and 40 generations.

Parasitism of lines varied widely from 0–95%, with the variation attributed to the need to maintain colonies using an aged *S. lepidus* population, particularly a problem in July–November of each year where there can be significant premature mortality of parasitised weevils. For this reason, it was necessary to maintain two to three replicates of the same line to minimise the risk that a line would be lost. The effect of *S. lepidus* age is reflected in the 33% average level of parasitism in summer-autumn, using newly emerged weevils, compared to ca 10% in winter-spring populations. Overall, quarantine based parasitism of *S. lepidus* by Irish *M. aethiopoides* over >20 parasitoid generations has averaged 25%.

Biological control of *S. lepidus* in New Zealand

The Irish strain of *M. aethiopoides* was first released as parasitised *S. lepidus* in early January 2006, with further releases in late January and February 2006. Subsequently, recoveries of the parasitoid have been made at the three sites with 26% parasitism already recorded in a small sample (n=23) collected in April 2006 from the initial site (P.J. Gerard, unpubl. data). Therefore, there is reasonable optimism that the Irish parasitoid biotype will provide successful biocontrol of the weevil, particularly based on the previous success with *M. aethiopoides* introduced from Morocco via Australia to control *S. discoideus* and *M. hyperodae* introduced from South America to control *L. bonariensis* (Goldson et al. 1990b, 1998).

This optimism is tempered by the knowledge that the probability of success as a biological control agent in exotic locations is greater when parasitism rates are high in the native range. Using published data from disparate field studies, Hawkins & Cornell (1994) found a significant relationship between maximum percentage parasitism in the native range and the probability of successful biological control in the exotic location. Of the 787 parasitoid introductions in their data set, none were successful unless there was at least 32% maximum parasitism observed in the pest's native range. Levels of parasitism of 80% and >60% were recorded for Moroccan *M. aethiopoides* and South American *M. hyperodae*, respectively, in their native ranges (Aeschlimann 1978; Goldson et al. 1990a). In contrast, a maximum of 7% parasitism was found in *S. lepidus* in Ireland. However, these low rates of parasitism in Ireland may reflect both the timing of the collections, and the possibility that there are undefined constraints on the parasitoid in Ireland that are absent in New Zealand. The rapid initial establishment of Irish *M. aethiopoides* in New Zealand *S. lepidus* populations indicates that the New Zealand pastoral environment is favourable for the parasitoid. The levels of parasitism at all release sites are being monitored at monthly intervals and by 2007 researchers will know if successful classical biocontrol of *S. lepidus* can be achieved.

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