

## POTENTIAL TO ENHANCE THE EFFICACY OF *MICROCTONUS HYPERODAE* LOAN

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### ABSTRACT

The parasitoid, *Microctonus hyperodae* Loan (Hymenoptera: Braconidae), and its host, Argentine stem weevil (*Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae)) were sampled, firstly, to quantify parasitoid mortality between the larval and adult stages during spring 1996 and, secondly, to measure the realised fecundity of two generations of parasitoid adults in summer 1996-1997. Survival of *M. hyperodae* larvae and pupae was low (<10%) and subsequent female density was low (<2/m<sup>2</sup>). Pasture management practices which increase survival of *M. hyperodae* larvae and pupae in spring could enhance suppression of *L. bonariensis*. Parasitoid females from the first half of the first adult generation were estimated to have laid <15% of their eggs, while females which emerged subsequently were estimated to have laid >65% of their eggs. This variation in realised fecundity was associated with changes in host density. Habitat manipulations that enable more of the early-emerging parasitoids to survive until host densities increase in early summer could enhance suppression of *L. bonariensis*.

**Keywords:** *Listronotus bonariensis*, *Microctonus hyperodae*, biological control, habitat manipulation, fecundity.

### INTRODUCTION

Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae), is a major pest of pasture in New Zealand (e.g. Goldson *et al.* 1998a). *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae) was introduced to New Zealand in 1991 for biological control of *L. bonariensis*. The parasitoid oviposits in the adult weevil, whereupon the parasitoid larva develops within the living host. *M. hyperodae* is parthenogenetic (Loan and Lloyd 1974) and proovigenic (Goldson *et al.* 1995; Phillips 1998). The parasitoid is now established at over 80 locations in the North and South Islands (M.R. McNeill pers. comm.).

This contribution makes a preliminary assessment of the potential to enhance the efficacy of *M. hyperodae* using habitat manipulation by, firstly, ascertaining mortality rates of immature *M. hyperodae* and, secondly, measuring the reproductive rates of *M. hyperodae* adults in the field.

### MATERIALS AND METHODS

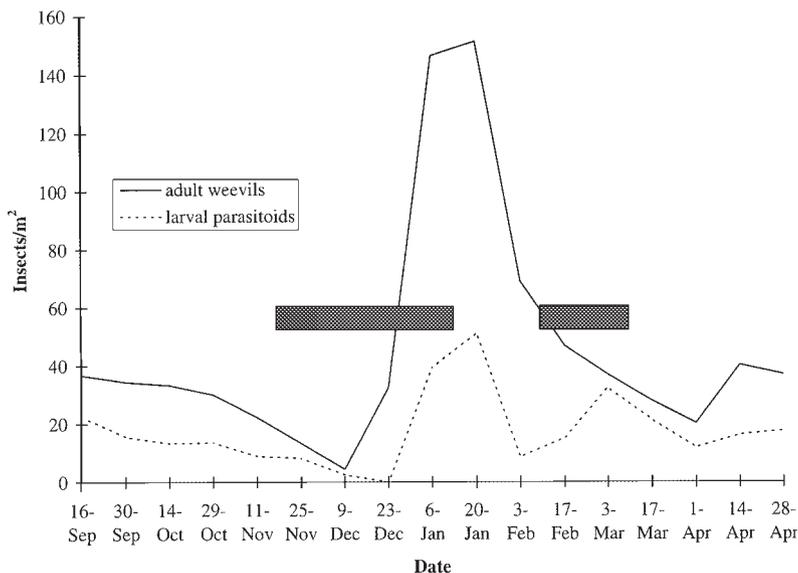
Sampling was conducted in one paddock (100 m x 100 m) at the AgResearch farm, Lincoln, between September 1996 and April 1997. This was the same paddock used previously to describe the bionomics of *L. bonariensis* and *M. hyperodae* (Goldson *et al.* 1998a and b). Estimates of the field densities of *L. bonariensis* adults and *M. hyperodae* larvae were made using the methods of Goldson *et al.* (1998a and b). In brief, adult weevils were extracted from turf samples (450 mm x 40 mm x 80 mm deep) taken approximately fortnightly using a flotation method (Proffitt *et al.* 1993). To provide additional weevils for assessment of parasitism, 50-70 weevils were also sampled every alternate week using a modified high-powered vacuum cleaner. Weevils recovered by both methods were dissected under 70% ethanol using a binocular microscope at 25 X magnification (Goldson and Emberson 1981) and the parasitoid stages present recorded.

*M. hyperodae* adults were sampled by collecting pasture litter each week along a 100 m transect across the paddock using a modified high-powered vacuum cleaner. A different transect was used each week. *M. hyperodae* adults were retrieved from the vacuum samples and stored at 4°C pending measurement of their egg loads. The eggs contained in the ovaries of each parasitoid were counted using the method of Phillips (unpublished) which involved removing the parasitoids' ovaries, staining and separating the eggs, then counting them at approximately 100 X magnification.

## RESULTS AND DISCUSSION

### Phenology of adult weevils

The *L. bonariensis* phenology observed in this study was consistent with that observed in the previous five years at the same site (Goldson *et al.* 1998a). *L. bonariensis* overwinters as adults. The density of adult weevils declines throughout winter and spring, reaching a minimum about mid December (Figure 1). Thereafter, weevil density begins to increase again with the emergence of first summer generation adults. Weevil density peaks in mid January, then declines rapidly until late March, whereupon a smaller peak in weevil densities indicates the emergence of second generation weevil adults.



**FIGURE 1: Density of *L. bonariensis* adults and *M. hyperodae* larvae at Lincoln between 16 September 1996 and 28 April 1997. The period of adult parasitoid occurrence is indicated by the horizontal bars.**

### Phenology of parasitoid larvae and adults

The *M. hyperodae* phenology observed in this study was consistent with that observed in the previous five years at the same site (Goldson *et al.* 1998b). *M. hyperodae* overwinters as 1st instar larvae and, during spring, these larvae develop, emerge from their hosts and pupate (Figure 1). The vacuum samples indicated that emergence of parasitoid adults recruited from overwintered larvae began in mid November and continued until mid January (Figure 1). These adult parasitoids laid eggs in first summer generation adult weevils and produced the first summer generation of parasitoid larvae

which was evident from late December 1996 until early February 1997 (Figure 1). Parasitoid adults recruited from this first summer generation of larvae occurred from 10 February until 10 March 1997.

### Densities of parasitoid larvae

Weevil dissection data indicated that overwintered parasitoid larvae began developing in mid September 1996 (no parasitoid pupae or adults were present at this time). The 16 September larval population density of 23/m<sup>2</sup> (Figure 1), therefore, provided an estimate of the total overwintered parasitoid population from which adult parasitoids would be recruited during early summer. Similarly, the peak density of parasitoid larvae of 51/m<sup>2</sup> on 20 January 1997 (Figure 1) provided an estimate of the total first summer generation parasitoid population (the *M. hyperodae* population comprised only larvae at this time as evidenced by adult parasitoids being absent from vacuum samples and parasitoid eggs being absent from weevil dissection samples). The larvae which occurred on 20 January 1997 (Figure 1), therefore, were the first generation progeny of the overwintered parasitoids.

### Evidence of high immature parasitoid mortality

On the same sampling dates that the overwintered parasitoid population of 23 larvae/m<sup>2</sup> increased to 51 larvae/m<sup>2</sup> in the subsequent generation, the adult weevil population increased from 37/m<sup>2</sup> to 151/m<sup>2</sup> (Figure 1). Using an estimated *M. hyperodae* mean potential fecundity of 67 (Phillips unpubl. data) and theoretical conditions of zero immature parasitoid mortality, nil migration, unlimited hosts and maximum oviposition by adult parasitoids, *M. hyperodae*'s overwintered population of 23 larvae/m<sup>2</sup> could have increased to 1541 larvae/m<sup>2</sup> in January (i.e. 23 parasitoid adults, multiplied by 67 eggs, equals 1541 larvae/m<sup>2</sup>). The observed January density of 51 parasitoid larvae/m<sup>2</sup>, therefore, indicates that one or both of the following possibilities occurred: a) There was very high mortality (maximum of 97%) of *M. hyperodae* larvae and pupae in September-November; b) There was very low net parasitoid reproduction in November-December (minimum of two offspring/female). The parasitoid egg load data, described below, indicated that mortality of *M. hyperodae* larvae and pupae was probably the most significant factor.

### Egg loads of parasitoid adults

Seventy eight parasitoid adults were collected during weekly sampling between 11 November 1996 and 21 April 1997 (Figure 2). These adults appeared in two discrete generations. The first was recruited from overwintered larvae and appeared in the samples from 18 November 1996 to 13 January 1997 (Figure 1). The mean egg load of these parasitoids was 49±4 eggs (n=43). The second generation of parasitoid adults was recruited from the first summer generation of parasitoid larvae and occurred in the samples from 10 February 1997 until 10 March 1997 (Figure 1). The mean egg load of these parasitoids was 31±4 eggs (n=35).

### Estimating parasitoid realised fecundity

Estimation of the number of eggs laid by *M. hyperodae* females was facilitated by the species' proovigenic reproduction (Goldson *et al.* 1995; Phillips 1998). Using the estimate of 67 eggs for potential fecundity, the eggs laid by each parasitoid prior to sampling was calculated as 67 minus the parasitoid's observed egg load. The mean number of eggs laid by parasitoids over their entire lifetimes (i.e. realised fecundity) was then calculated based on three assumptions. The first was that parasitoid potential fecundity did not vary with time, the second was that the rate of parasitoid oviposition was independent of parasitoid age and the third was that, on average, parasitoid adults were sampled when they were half way through their lifespans. These assumptions meant that realised fecundity could be calculated as two times the number of eggs laid prior to sampling, with an upper limit of 67 eggs.

### Mean realised fecundity of the November-January parasitoids

The egg loads for parasitoids recruited from overwintered larvae (November-January; Figure 1) indicated that, on average, each *M. hyperodae* female had laid 18 eggs prior to being sampled (i.e. potential fecundity of 67, minus egg load of 49, equals 18 eggs laid) which, as described above, was doubled to derive an estimated mean realised fecundity of 36 eggs/parasitoid.

### Estimating the population density of adult parasitoids

Using the estimated mean realised fecundity of 36 eggs for *M. hyperodae* adults recruited from overwintered larvae, and assuming zero post-oviposition mortality of these eggs, the subsequent summer generation of parasitoid larvae, which reached a density of 51/m<sup>2</sup>, must have been produced by 1.4 *M. hyperodae* adults/m<sup>2</sup> (i.e. 51 larvae/m<sup>2</sup>, divided by 36 eggs/female, equals 1.4 *M. hyperodae* adults/m<sup>2</sup>). However, the assumption of zero post-oviposition mortality of parasitoid eggs used in deriving the above estimate of adult parasitoid density is probably unrealistic as there are two sources of egg mortality apparent in early summer. The first is that parasitoid adults present from 18 November to 9 December probably laid eggs in overwintered weevils (Figure 1). These weevils were liable to die before mid December (Goldson *et al.* 1998a), leaving insufficient time for the parasitoid eggs to complete their development. Such parasitoid eggs, therefore, could not contribute to the first summer generation of parasitoid larvae which became evident on 23 December (Figure 1). The effect of this mortality factor can be estimated by assuming that all of the 14 unparasitised weevils/m<sup>2</sup> present on 16 September (Figure 1) became parasitised but, because of the imminent death of these weevils, the parasitoid eggs laid in them did not contribute to the first summer generation of parasitoid larvae. The second source of *M. hyperodae* mortality is that females sometimes oviposit in weevils that are already parasitised (Goldson *et al.* 1998b), although only one immature parasitoid can survive in each host (Loan and Lloyd 1974). Between 18 November and 14 January, there was a mean of 1.4 supernumerary parasitoid larvae/m<sup>2</sup>.

The 14 parasitoid eggs/m<sup>2</sup> assumed to have died in overwintered weevils, plus the 1.4 parasitoid eggs/m<sup>2</sup> estimated to have died due to superparasitism, plus the 51 parasitoid larvae/m<sup>2</sup> observed on 20 January (Figure 1), suggest that *M. hyperodae* females recruited from overwintered larvae laid 66 eggs/m<sup>2</sup>. Using the estimated mean realised fecundity of 36 eggs/female, the 66 eggs/m<sup>2</sup> must have been laid by 1.8 *M. hyperodae* adults/m<sup>2</sup> (i.e. 66 eggs/m<sup>2</sup>, divided by 36 eggs/female, equals 1.8 females/m<sup>2</sup>).

### Comparison of the estimate of adult parasitoid density with prediction of a model

The estimated November-January parasitoid adult density of 1.4/m<sup>2</sup> to 1.8/m<sup>2</sup> was compared to an estimate derived from the model of Barlow *et al.* (1993) for *M. hyperodae* searching efficiency. This model predicted parasitoid adult density from the proportion of hosts parasitised, and was based on the results of field cage experiments which measured parasitism over a range of *M. hyperodae* and *L. bonariensis* densities. On 20 January 1997, 34% of *L. bonariensis* adults contained a *M. hyperodae* egg or larva (Figure 1). Using this figure for proportion of hosts parasitised, the model (Barlow *et al.* 1993) estimated that this parasitism resulted from oviposition by 1.9 *M. hyperodae* adults/m<sup>2</sup> and, therefore, corroborated the analysis described above.

### Immature parasitoid mortality during spring

The estimated 1.4-1.8 *M. hyperodae* adults/m<sup>2</sup> were recruited from an overwintered larval population of 23/m<sup>2</sup>, suggesting that only 6-8% of overwintered parasitoid larvae present on 16 September 1996 reached the adult stage (i.e. 1.8 *M. hyperodae* adults/m<sup>2</sup>, divided by 23 overwintered *M. hyperodae* larvae/m<sup>2</sup>, times 100, equals 8%).

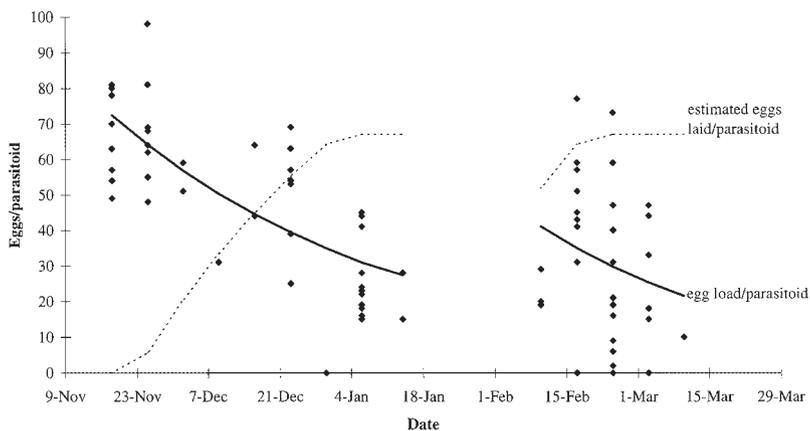
### Causes of immature parasitoid mortality during spring

Reasons for the estimated 92-94% parasitoid mortality which occurred between the overwintered larval generation and the subsequent adult generation are unknown. In spring, it is possible that some parasitised weevils die before parasitoid larvae can complete their development and emerge. This could be estimated by collecting weevils in September, maintaining them under conditions simulating those of the field, retrieving weevils as they die, and measuring the proportion of dead weevils that contain a parasitoid. It appears, however, that it would be difficult to develop a method to reduce such a source of mortality.

The 92-94% parasitoid mortality could also have been due to mortality of pre-pupae and/or pupae; *M. hyperodae* probably pupates underneath plant litter on the soil surface. There are numerous possible causes of mortality of pre-pupal and pupal *M. hyperodae* including low temperatures (Barlow *et al.* 1994), low humidity (M.R. McNeill pers. comm.), trampling by stock, disease, flooding, predation by staphylinids (this has been observed in the laboratory; Goldson unpubl. data) and predation by other arthropods. Of these, temperature, humidity and stock trampling would appear most amenable to manipulation. For example, temperature and humidity at the soil surface (and, therefore, pupal survival) are likely to be influenced by sward height and density, while pupal mortality due to stock trampling could be influenced by grazing management.

**Reproductive rate of *M. hyperodae* adults**

For the two generations of adult parasitoids, negative exponential curves were fitted by regression with Poisson errors and a logarithmic link function (Green and Silverman 1994) to the counts of eggs contained by each parasitoid on each sampling date (solid lines, Figure 2). Probabilities were calculated using an approximate F test based on ratios of mean deviances. From the regression lines in Figure 2, it can be seen that the first emerging parasitoid adults which occurred on 14 November contained a mean of 72 eggs. Thereafter, egg loads declined such that, by the end of the first adult generation on 14 January, the mean egg load was 27 ( $P < 0.001$ ; Figure 2). The second generation of parasitoid adults displayed a similar decline in egg load with time, although the mean egg load, both of the first adults on 10 February and the last adults on 10 March, was lower (41 and 21 eggs respectively,  $P > 0.05$ ; Figure 2). The egg load data were used to estimate, as described previously, the mean realised fecundity of the parasitoids on each sampling date (dotted curves, Figure 2). This estimation of variation in realised fecundity would become very biased if the average lifespan of *M. hyperodae* adults comprised a very large proportion (e.g. 50%) of the total sampling period. It seems likely that adults live less than two weeks in the field, since their longevity under ideal conditions in the laboratory was 17 days (Hodgson *et al.* 1993; Phillips 1998). Adult lifespans, therefore, were likely to have comprised less than 10% of the total sampling period.



**FIGURE 2:** Egg load of each of the 78 parasitoids collected from 11 November 1996 to 21 April 1997. The solid line shows the exponential decay regression curve fitted to the data and was used to provide an estimate of the parasitoid population’s mean egg load on each sampling date. The dotted line shows the parasitoid population’s estimated mean realised fecundity on each sampling date.

### Potential to enhance the efficacy of *M. hyperodae* adults

As can be seen from Figure 2, adult parasitoids representing the second half (mid December - mid January) of the generation recruited from overwintered larvae laid 65-100% of their eggs. This high realised fecundity was associated with the emergence of first summer generation adult weevils which began in mid December; adult weevil densities averaged approximately 90/m<sup>2</sup> from mid December to mid January (Figure 1). Similarly, parasitoids representing the first summer generation of adult parasitoids (mid February - mid March) laid 70-100% of their eggs (Figure 2). This high realised fecundity was also associated with moderately high, though declining, host densities which averaged 37 weevils/m<sup>2</sup> from mid February to mid March (Figure 1). Based on the estimation that adult parasitoids from the latter half of the generation recruited from overwintered larvae, as well as the whole of the subsequent first summer generation, laid 65-100% of their eggs (Figure 2), there appears to be little potential to improve their efficacy by habitat manipulation.

Parasitoids which represented the first half (mid November to mid December) of the generation recruited from overwintered larvae, however, laid less than 15% of their eggs (Figure 2). This low realised fecundity was associated with a senescing, overwintered host population that had an average density of 13/m<sup>2</sup> from mid November to mid December (Figure 1). Moreover, the relatively few eggs laid by these parasitoids probably contributed little to parasitoid reproduction since the overwintered hosts available for parasitism were liable to die before parasitoid larvae could complete their development. It appears, therefore, that a method of increasing the longevity of parasitoids which emerge between mid November and mid December, so that they survive until host densities increase, could enhance *M. hyperodae*'s impact on *L. bonariensis*. For example, in very approximate terms, increasing the number of parasitoid adults surviving until mid December by 20%, when parasitoid realised fecundity was 44 eggs (Figure 2), should result in a 31% increase in parasitism on 20 January. That is, a 0.2 increase in adult parasitoid density, multiplied by 1.8 parasitoid adults/m<sup>2</sup>, multiplied by 44 eggs, equals 16 additional larvae/m<sup>2</sup>; 16 larvae/m<sup>2</sup> divided by 51 larvae/m<sup>2</sup> (as measured on 20 January 1997; Figure 1) equals a 31% increase in parasitism.

To exploit this potential to enhance suppression of *L. bonariensis*, it is possible that *M. hyperodae* adult longevity could be increased in the field by the timely provision of suitable liquid foods. In the laboratory, the longevity of *M. hyperodae* females was increased from 4 days to 17 days when they had access to liquid food (Hodgson *et al.* 1993; Phillips 1998), although their potential fecundity was not increased by this treatment (Phillips 1998). Research is underway to ascertain whether or not *M. hyperodae* females are already successfully foraging for food in New Zealand farm environments. If not, then the suitability of possible food sources (e.g. nectar bearing plants) will be examined and the most appropriate sources tested in the field.

### Implications for life history theory

The preliminary data presented in this contribution have relevance to the development of life history theory. Parasitoid fecundity has often been assumed to be limited by the rate at which hosts are encountered, and it has only recently been recognised that parasitoid fecundity could also be limited by egg load, although data supporting this latter prediction are still rare (Heimpel and Rosenheim 1998). The high realised fecundity of *M. hyperodae* after mid December (Figure 2) indicates that it could become egg depleted before the end of its lifespan. If *M. hyperodae* females do become egg limited relatively frequently under New Zealand conditions, then there could be strong selection pressure favouring higher egg loads.

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## REFERENCES

- Baggen, L.R. and Curr, G.M., 1998. The influence of food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Bio. Cont. 11*: 9-17.
- Barlow, N.D., Goldson, S.L. and McNeill, M.R., 1994. A prospective model for the phenology of *Microctonus hyperodae* (Hymenoptera: Braconidae), a potential biological control agent of Argentine stem weevil in New Zealand. *Biocon. Sci. and Tech. 4*: 375-386.
- England, S. and Evans, E.W., 1997. Effects of pea aphid (Homoptera: Aphididae) honeydew on longevity and fecundity of the alfalfa weevil (Coleoptera: Curculionidae) parasitoid *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae). *Env. Ent. 26*: 1437-1441.
- Goldson, S.L. and Emberson, R.M., 1981. Reproductive morphology of the Argentine stem weevil, *Hyperodes bonariensis* (Coleoptera: Curculionidae). *N.Z. J. Zoo. 8*: 67-77.
- Goldson, S.L., McNeill, M.R., Proffitt, J.R. and Hower, A.A., 1995. An investigation into the reproductive characteristics of *Microctonus hyperodae* (Hymenoptera: Braconidae), a parasitoid of *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) *Entomophaga 40*: 413-426.
- Goldson, S.L., Proffitt, J.R. and Baird, D.B., 1998a. The bionomics of *Listronotus bonariensis* (Coleoptera: Curculionidae) in Canterbury, New Zealand. *Bull. Ent. Res.*: in press.
- Goldson, S.L., Proffitt, J.R. and Baird, D.B., 1998b. Establishment and phenology of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae) in the Canterbury region of New Zealand. *Env. Ent.*: in press.
- Green, P. and Silverman, B., 1994. Nonparametric regression and generalized linear models. London: Chapman & Hall.
- Heimpel, G.E. and Rosenheim, J.A., 1998. Egg limitation in parasitoids: A review of the evidence and a case study. *Bio. Cont. 11*: 160-168.
- Hodgson, D.J., Potter, M.A., Lovei, G.L., McNeill, M.R. and Goldson, S.L., 1993. The effects of water, glucose and pollen as dietary components on the longevity of adult *Microctonus hyperodae* (Hymenoptera: Braconidae, Euphorinae), a parasitoid of *Listronotus bonariensis* (Coleoptera: Curculionidae). *Proc. 6th Australasian Conf. Grass. Invert. Ecol.*: 384-390.
- Loan, C.C. and Lloyd, D.C., 1974. Description and field biology of *Microctonus hyperodae* Loan n. sp. (Hymenoptera: Braconidae; Euphorinae) a parasite of *Hyperodes bonariensis* in South America (Coleoptera: Curculionidae). *Entomophaga 19*: 7-12.
- Phillips, C.B., 1998. Influence of liquid food on fecundity and longevity of *Microctonus hyperodae* Loan. *Proc. 51st N.Z. Weed and Pest Control Conf.*: this volume.
- Proffitt, J.R., Ferguson, C.M., McNeill, M.R., Goldson S.L., Macnab, H., and Barratt, B.I.P., 1993. A comparison of sampling methods for adult *Listronotus bonariensis* (Coleoptera: Curculionidae). *Proc. 6th Australasian Conf. Grass. Invert. Ecol.*: 67-72.