THE EFFECT OF COOL STORAGE ON THE SURVIVAL OF THE ARGENTINE STEM WEEVIL PARASITOID, *MICROCTONUS HYPERODAE* LOAN.

S.L. GOLDSOON, M.R. MCNEILL, J.R. PROFFITT and D.B. BAIRD

MAF Technology, PO Box 24, Lincoln, Canterbury

SUMMARY

Experiments were conducted to examine the effect of cool storage on *Microctonus hyperodae* Loan, a parasitoid of the Argentine stem weevil, *Listronotus bonariensis* (Kuschel). When the parasitoid was maintained as larvae within infected weevils at 6°C, there was high mortality both during and after storage. Storage of up to 4 weeks had the least impact on subsequent prepupal emergence, although, even after 8 weeks cool storage, worthwhile production of parasitoid pupae was still achievable. An unexplained observation was the significantly higher mortality amongst the uninfected portion of the parasitoid-exposed population compared to the parasitoid-free controls.

Keywords: *Listronotus bonariensis*, parasitoid, cool storage, viability.

INTRODUCTION

The parasitoid *Microctonus hyperodae* Loan was introduced into quarantine in New Zealand in 1989-90 (Goldson *et al* 1990) for assessment as a biological control agent against Argentine stem weevil *Listronotus bonariensis* (Kuschel) (Loan and Lloyd 1974). After a year of quarantine-based evaluation, the host range of the species was found to be sufficiently narrow (Goldson *et al* 1992) and, accordingly, permission to release was granted.

For technical and biological reasons *M. hyperodae* was released in infected weevils. To this effect, in the winter of 1991 a mass-rearing programme was commenced such that equal numbers of each of seven imported 'ecotypes' were reared. These ecotypes had earlier been collected from a diverse range of South American geographical and climatic zones (Goldson *et al* 1990). Equal numbers of each parasitoid ecotype were systematically liberated at eight sites in the northern half of the North and South Islands and in Otago. This approach presented the opportunity for later analysis of patterns of ecotypic establishment in different climate zones.

The process of mass-rearing of *M. hyperodae* resulted in nine releases of a total of c.100 000 infected weevils being made between April and October 1991. Such a scale of rearing necessitated that some parasitised weevils were maintained in 6°C storage until equal numbers of each ecotype for that particular release were produced. Prior to this, it had been ascertained that the parasitoid was able to develop on weevils that had been maintained at 10°C in storage (Goldson and Proffitt 1990). This contribution describes the steps taken to ascertain that *M. hyperodae* did not undergo excessive mortality during storage and that when reintroduced to temperatures above the development threshold, pupation and subsequent adult eclosion occurred normally.

MATERIALS AND METHODS

Weevils used in this experiment were sweep-netted from ryegrass dominant pasture (cv. Manawa) in Canterbury on 1 May 1991 and thereafter stored at 10°C and 16:8 L:D photoperiod for 4 weeks until the commencement of the experiment.

To achieve parasitism, four replicates of each of seven parasitoids of mixed ecotypic origin (Brazil, Uruguay, Rio Negro and La Serena) were placed with c.200 weevils for 9 days in clear plastic 8 litre containers maintained at 20°C, in a L:D 16:8 photoperiod. As controls, three identical containers each with c.250 weevils were

Proc. 45th N.Z. Plant Protection Conf. 1992: 210-214

established under the same conditions but in the absence of parasitoids. Weevils were provided with fresh ryegrass that was replaced on day four. At the onset of the experiment 46 weevils were sampled and dissected to determine their reproductive status and the level of any opportunistic field parasitism by *Microctonus aethioides* Loan. Similarly after the completion of the inoculation phase, 100 weevils were sampled and dissected to determine the approximate level of incurred parasitism.

After 9 days, the parasitised weevils and controls were stored at 6°C and L:D 16:8 photoperiod. On eight consecutive weekly occasions, cadavers were removed from the control and parasitoid-exposed populations, counted and dissected to ascertain the incidence of parasitism. At the same time c.70 weevils were taken from both populations, placed in gauze-floored holding cages (Goldson and Proffitt 1990) and transferred to 20°C, 16:8 L:D photoperiod. Under these conditions the populations were checked weekly for prepupal emergence and weevil mortality. Where possible the cadavers were dissected to ascertain levels of parasitism. Once prepupal emergence was complete, surviving weevils were dissected to determine the level of parasitism. Pupae were incubated at 20°C and survival rates ascertained.

Cumulative mortality of the different populations was determined using the Kaplan-Meier estimator of the survivorship curve (Cox and Oakes 1984), which allows for the censoring which occurred when samples were taken for laboratory analyses. The standard errors for the Kaplan-Meier curves were generated by bootstrap resampling (Hinkley 1988) of the observed deaths. Where the data were proportions, the assumption was made that the data came from a binomial distribution and exact confidence limits were calculated from the number of samples and the estimated proportions.

RESULTS AND DISCUSSION

The background infection of the field-collected Argentine stem weevil population by *M. aethioides* was 5%. After inoculation, parasitism by *M. hyperodae* was found to be 54% in an initial sample of 100 weevils. A better estimate of the initial parasitism after inoculation was obtained at the end of the trial when 50% of the 467 weevils dissected from the inoculated population were found to be parasitised.

During cool storage there was significantly ($P < 0.001$) higher mortality in the parasitoid-exposed population (Fig. 1) such that by the eighth week of storage mortality in the control population had reached 53% compared with 75% in the parasitoid-exposed population. The parasitoid-exposed population showed pronounced mortality between weeks four and six (Fig. 1).

Dissection of cadavers that appeared during cool storage in the first and second week showed that these were only 20% and 14% parasitised respectively (Fig. 2); considerably less than the overall level of parasitism of 50%. However these percentages were based on small samples ($n=5,7$ respectively) and so the differences were not statistically significant. Thereafter the incidence of parasitism amongst the cadavers was c.54% indicating an absence of notable differential mortality between the parasitised and unparasitised populations (Fig. 2).

After removal from cool storage and transfer to 20°C there was significantly ($P < 0.001$) higher mortality (approximately double) amongst the parasitoid-exposed population compared with the control, irrespective of the time that they had earlier spent in cool storage (Fig. 3). The cadavers were found to be uniformly parasitised at c.72% indicating that there was higher mortality amongst the infected individuals.

Using the dissection data from the cadavers and the estimated initial number of parasitised weevils, the cumulative mortality of the parasitised and non-parasitised sub-groups of the infected population was calculated (Fig. 4). Both the parasitised and non-parasitised sub-groups of the parasitised population showed significantly ($P < 0.01$) higher mortality (79% and 71% respectively) by the end of the experiment than the control population. The difference of 8% between the two subgroups was not statistically significant.

The estimation process used to obtain the cumulative mortality of parasitised and non-parasitised sub-groups also yielded an estimate of the proportion of weevils transferred each week to the laboratory which will yield prepupae. The estimated.

Fig. 1: Cumulative Argentine stem weevil mortality amongst parasitoid-exposed and control populations.

Fig. 2: The proportion of Argentine stem weevil cadavers parasitised and estimated and observed prepupal emergence vs storage duration.

Fig. 3: Argentine stem weevil mortality in parasitoid-exposed and control populations after removal from cool storage and transfer to the laboratory vs storage duration.

Fig. 4: Cumulative Argentine stem weevil mortality amongst the parasitised and non-parasitised sub-groups of the parasitoid-exposed population, compared to the control population.

and observed prepupal yield are shown in Figure 2. The estimated yield of prepupae is relatively constant at c.50% due to the parasitised and non-parasitised sub-groups having similar mortality. The eventual yield of prepupae as a percentage of the surviving weevils in the storage cages declined from c.53% after 1 week's cool storage to 23% after 8 weeks (Fig. 5). The decline in yield became particularly pronounced after week four. Such a result suggests that there was deterioration in the vigour of stored parasitoid larvae. However, there was no parallel trend in the appearance of malformation in larvae dissected from weevils alive at the completion of the laboratory experiment. Nonetheless, atypical malformations of larval parasitoids did occur in the form of an apparent separation of dermal layers to produce a form of 'blistering'. There was no degradation in pupal survival, with all pupae recovered showed typical survival rates of 83 - 97%.

An unexplained facet of this experiment was the observation in the storage population of a uniformly significantly higher level of mortality amongst weevils in the unparasitised portion of parasitoid-exposed populations compared with those in the control. The consistency of these results and other unpublished observations suggests that this was a real effect. This may be attributable to some kind of parasitoid-induced behavioral modification amongst the infected weevils. Alternatively the parasitoids may sting the weevils and inject toxin without actually laying an egg which in turn could contribute to mortality.

CONCLUSION

The data in this study suggests that there was a decline in *M. hyperodae* viability when cool-stored as first instar larvae although even after 8 weeks, populations were still usefully viable for field release. During the mass production of *M. hyperodae*, a small proportion of the parasitised weevils were stored for up to 5 weeks although in general the bulk of the release population was in cool storage for less than 4 weeks thus avoiding any significant loss.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Ms S. Clemett for her skilful dissection of the weevil samples.

REFERENCES

- Cox, D.R. and Oakes, D., 1984. Survival Models and Data Analysis. Chapman and Hall, London.

- Goldson, S.L., McNeill, M.R., Stufkens, M.W., Proffitt, J.R., Pottinger, R.P. and Farrell, J.A., 1990. Importation and quarantine of *Microctonus hyperodae*, a South American parasitoid of Argentine stem weevil. *Proc. 43rd N. Z. Weed and Pest Control Conf.*: 334-338.
- Goldson, S.L., McNeill, M.R., Phillips, C.B. and Proffitt, J.R., 1992. Host specificity testing and suitability of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae) as a biological control agent of *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) in New Zealand. *Entomophaga* (in press).
- Goldson, S.L. and Proffitt, J.R., 1990. The effect of storage on the ability of Argentine stem weevil to sustain development of the parasitoid *Microctonus hyperodae*. *Proc. 43rd N. Z. Weed and Pest Control Conf.*: 339-342.
- Hinkley, D.V. 1988. Bootstrap methods. *J. Royal Stat. Soc. B*, 50: 321-337.
- 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.