

THE POTENTIAL OF PARASITOID STRAINS IN BIOLOGICAL CONTROL; OBSERVATIONS TO DATE ON *MICROCTONUS* SPP. INTRASPECIFIC VARIATION IN NEW ZEALAND

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ABSTRACT

Over the last 15 years New Zealand researchers have been actively involved in the classical biological control of forage weevil pests. In the 1980s the lucerne pest *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) was successfully suppressed by the parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae). Since 1990 an additional programme has been developed to examine the impact of the recently introduced parasitoid *Microctonus hyperodae* (Hymenoptera: Braconidae) on New Zealand's worst ryegrass pest, the Argentine stem weevil *Listronotus bonariensis* (Kuschel)). The imported *M. hyperodae* founder populations were collected from a wide range of ecoclimatic zones.

During both of the programmes the importance of ecotypes has become increasingly apparent. The New Zealand *M. aethiopoides* population revealed phenological behavioural patterns quite different from those observed in Mediterranean Europe, North America and Australia. As a result of these observations, part of the current research into *M. hyperodae* has been developed to specifically explore any inherent ecotypic differences and their implications.

This contribution reviews progress to date in researching ecotypic differences and comments on the potential of ecotypes in classical biological control. This is with particular reference to recently developed DNA-based techniques that permit differentiation between populations at the subspecific level.

INTRODUCTION

Biological control has been sporadically subjected to criticisms such as those of Krebs (1972) who considered it to be akin to gambling. This may well be true when benefits to agricultural production are expected to be provided as quickly as possible and at minimum expense. This low cost approach however reduces the potential to gain scientific understanding from biological control initiatives. Scientific investigation into the principles of biological control is the only way to impart certainty into the approach's decision-making and optimisation (Greathead, 1986) and thus reduce the gambling element.

Biological control research is closely linked to a range of science areas and has for some time been recognised as a branch of applied ecology more than agriculture (Krebs, 1972). Agroecosystems cannot be treated in isolation from neighbouring natural areas partly because introduced organisms are likely to invade all suitable habitats, agricultural or otherwise (Howarth, 1991; Ferguson *et al.*, in press).

New Zealand agriculture provides simplified habitats that are well-suited to the study of biological control. The New Zealand indigenous arthropod fauna comprises c. 20 000 spp. that have been geographically isolated for over 100M years and is therefore unique (Emberson, 1994). In contrast, pest species and their control agents are often exotic and therefore ecologically remote from the native fauna. Many northern hemisphere plants have also been introduced into New Zealand and are similarly differentiated from the native fauna. Because of these circumstances many of the confounding effects such as have been discussed by Waage (1990) that arise from interactions between numerous closely related species can be avoided. Two New Zealand studies based on exotic *Microctonus* spp. parasitoids illustrate how this situation has allowed the beginning of analysis into the role and impact of intraspecific variation in biological control. Some of the approaches, findings and opportunities are presented in this contribution.

THE IMPORTANCE OF INTRASPECIFIC VARIATION IN BIOLOGICAL CONTROL

Since the turn of the century, species have ceased to be regarded as something fixed and uniform but are now considered to be polytypic comprising many subspecies and local populations (Mayr *et al.*, 1953). Arising from this, there has been a proliferation of terms to describe these entities such as subspecies, race, microspecies, ecotype, variant, clone, line, strain and biotype (Steiner, 1994). In addition to such diverse terminology, the situation has been confounded further by the lack of obvious fixed criteria for discrimination between subspecific groups (Gonzalez *et al.*, 1979). For the purpose of this contribution, subspecific groups will hereafter be referred to as 'strains' and when discussed the general assumption will be that their genotypes differ from those of other groups within the species being considered. These issues and the associated genetics have recently been reviewed in relative depth by Narang *et al.* (1994).

It may be argued that in recent years there has been increasing interest in strains in biological control. Such a development has probably arisen in part from the improving quality and availability of biological control theory (e.g. Mackauer *et al.*, 1990; Narang *et al.*, 1994; Godfray, 1994). The growth in interest has probably also been related to increasing awareness that exotic biological control agents can threaten non-target native species; particularly those that are insular in their distributions (Howarth, 1991). Ironically, a wide host range was seen to be desirable as it allowed a biological control agent to exist on other species when the target species were scarce (e.g. DeBach & Bartlett, 1964; Watt, 1965). A direct consequence of the increasing concern about the environmental impacts of biological control agents has been a reduction in the number of acceptable biological control species. The ability to make useful multiple introductions as discussed by Huffaker *et al.* (1971) is therefore now considerably reduced and there is a greater imperative than ever for suitable species to be used to their maximum potential. Fortunately, this need for improved precision in recognition, re-examination, selection and exploitation of suitable agents has coincided with the advent of DNA-based discriminatory methods. Analyses of some sequences of ribosomal RNA and transfer RNA can be particularly useful in distinguishing between parasitoid strains (Narang *et al.*, 1994).

Roush (1990) examined in a general way the importance of intraspecific variation to biological control. He suggested that, to collect a founder population of a biological control agent that comprises a reasonable expression of a species' allelic diversity, 20 individuals may form an absolute minimum while populations of over 100 probably offer little additional advantage. The need to maintain genetic variation in the importation and colonisation processes was also emphasised. This included examination of how to best release different sexually reproducing strains collected from separate sites. The extent of understanding remains inconclusive and it is still debatable as to whether different strains should be released separately at different sites, separately at the same site or hybridised prior to release (Roush, 1990). Roush (1990) also pointed out that there are few examples in the literature where genetic variation has clearly influenced the success of biological control agents; efforts to demonstrate the importance of genetic variation are complicated by the need to discern between phenotypic and genotypic variation in biological control success.

The probable importance of strains can be illustrated using two biological control programmes involving *Microctonus* Wesmael spp. parasitoids in New Zealand. While neither of these programmes necessarily represents uniquely inspired planning or execution, both have had a notable measure of success with respect to pest suppression.

Research with *Microctonus aethiopoides*

This is an example of a parasitoid species which has a very variable biological control performance in different regions and/or with different host species.

The lucerne (*Medicago sativa* L.) pest *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) was first discovered in New Zealand in 1974. It rapidly became a severe pest in this country, particularly in light stony soils that preclude any build up of mineralised nitrogen because of leaching (Goldson *et al.*, 1985). Massive spring *S. discoideus* larval populations of up to 4000 m⁻² frequently destroyed all of the plant's nitrogen-fixing rhizobial root nodules (Goldson *et al.*, 1984; Goldson *et al.*, 1985), often resulting in mid-season production losses of up to 50% (Goldson *et al.*, 1985). This was generally manifest as an abrupt cessation of growth once a threshold of 1100-2000 larvae m⁻² had been exceeded (Goldson *et al.*,

1985). It was postulated that these thresholds occurred due to loss of the crop's photosynthetic ability when demand for nitrogen exerted by re-establishing nodules exceeded that contributed by any remaining intact nodules (Goldson *et al.*, 1988a). The monocultural nature of lucerne stands made these damage thresholds very easy to establish.

The detailed nature of the study on *S. discoideus* and its impacts subsequently permitted the construction of full population dynamics analyses using several years' data (Goldson *et al.*, 1988b), which allowed the impact of the biological control agent *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) to be measured. *M. aethiopoides* was introduced into New Zealand in 1982 from Morocco and Greece via South Australia (Aeschlimann, 1983). While this parasitoid had earlier shown indifferent pest suppression elsewhere (e.g. Hopkins, 1985), it was successful in New Zealand (Goldson *et al.*, 1990; Barlow & Goldson, 1993). This occurred because 3% of the Canterbury *M. aethiopoides* population did not enter sympatric aestivation with their hosts as has been consistently reported in other countries (e.g. Abu & Ellis, 1976; Cullen & Hopkins 1982), but continued to develop in pre-aestivatory weevils. Due to this atypical development, infected weevils became stranded in the lucerne fields unable to migrate out to the aestivation sites after their spring emergence (Goldson *et al.*, 1984). This resulted in adult wasps eclosing and remaining in the lucerne ready to attack further weevils as soon as they emerged. Later, after their autumnal return, unparasitised post-aestivatory populations were also immediately 'ambushed' by the pre-existing field populations of atypically-developed *M. aethiopoides* adults (Goldson *et al.*, 1990). The combined effect of such patterns of attack resulted in an average 60% *S. discoideus* parasitism after aestivation but prior to the bulk of weevil egg-laying (Goldson *et al.*, 1990). This is far higher than has been found elsewhere (e.g. Hopkins, 1985) and as such has been shown to be essential to the success of the programme (Barlow & Goldson, 1993) in spite of compensatory effects of density-dependent survival by the larvae (Goldson *et al.*, 1988b). The reason for this difference in *M. aethiopoides* behaviour compared to that observed elsewhere remains unknown. It is notable however that parasitoids from the same source did not show the same characteristics in Australia (Cullen & Hopkins, 1982).

The atypical New Zealand *M. aethiopoides* population behaviour is paralleled by variations in the species' biology noted elsewhere. Loan and Holdaway (1961) observed significantly different levels of survival amongst French and Moroccan strains of *M. aethiopoides* on *S. cylindricollis* Fahraeus. Studies in the United States have similarly found *M. aethiopoides* to be highly active against alfalfa weevil (*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)) (Dysart & Day, 1976) but not against *S. hispidulus* Fabricius or *S. cylindricollis* (Day *et al.*, 1971).

Primitively, *M. aethiopoides* is distributed throughout Europe including Sweden, France, Croatia, Rumania, Russia, Ukraine and Uzbekistan (Loan, 1975) as well as the Mediterranean including Morocco, Greece, Algeria, Tunisia, Portugal, Spain and Italy (Aeschlimann, 1980). Given such a wide distribution, it may be argued that at least some of the observed variation may be based on genetic differences and to this effect, considerable work based on morphology and behaviour has already been done (Adler & Kim, 1985; Sundaralingam, 1986). However, at least some of the variation that has been attributed to strains could be phenotypic effects arising from varied host associations (Phillips *et al.*, 1993), as have been found in other parasitoid species (e.g. Janzon, 1986; Johnson *et al.*, 1987).

No published DNA-based analyses have been conducted to analyse the genetic diversity of *M. aethiopoides*. Such an approach would assist in understanding the basis for behavioural and morphological differences in populations. It may be argued that, had there been a better understanding of the implications of *M. aethiopoides* strains at the outset, more appropriate release strategies could have been developed. Notwithstanding this however, the application of new techniques for subspecific differentiation could still greatly improve the potential of *M. aethiopoides*. In the first instance this would probably involve the collection of different European and Mediterranean populations of *Sitona* spp. and *Microctonus* spp. for DNA analysis. In this way, the New Zealand, Australian and American *Microctonus* spp. and *Sitona* spp. populations would be able to be compared to the original geographical populations, and an understanding of strainal basis for the variable performance of *M. aethiopoides* developed. Such a study could also reveal whether the relatively broad host range of the New Zealand *M. aethiopoides* population (Ferguson *et al.*, 1994) is genetically based. It can be speculated that these parasitoids may be showing some kind of subspecific 'hybrid vigour' and insight into this would help resolve some of the issues raised by Roush (1990).

Research with *Microctonus hyperodae*

This programme represents an experimental analysis of the significance of intraspecific variation in the context of a practical biological control effort.

Study into the biological control of Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) (Coleoptera: Curculionidae) using a virtually unknown parthenogenetic parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae) (Loan & Lloyd, 1974) permitted some of the theoretical questions raised during the *M. aethioides* programme to be tackled experimentally from the start. It was intended that the study involving *M. hyperodae* should be in part a response to the challenge of workers like Waage (1990) and Roush (1990) who have lamented the lack of systematic field experimentation into biological control. Waage (1990) pointed out how little study there has been using replicated introductions of different agents in different regions without appropriate controls, while Roush (1990) noted that with a little creativity it should be possible to find situations where experimental releases could be arranged without sacrificing the urgency for solving pest problems.

Background

L. bonariensis apparently established in New Zealand without its suite of natural enemies at the turn of the century and is now recognised as New Zealand's worst ryegrass pest. In particular, the tiller-mining larval stages cause insidious damage that is often characterised by lack of recovery of pasture after summer drought (e.g. Whatman, 1959) and/or change in composition to clover or weed dominance (e.g. May, 1961; Goldson & Trought, 1980). Pesticides have not been cost-effective against *L. bonariensis* and much of the current management practice of this species depends on the use of plant resistance based largely on ryegrass infected with the endophyte fungus *Acremonium lolii* Latch, Christensen and Samuels (e.g. Prestidge *et al.*, 1982; Prestidge & Ball, 1991). *A. lolii* produces toxins in the grass which deters feeding by *L. bonariensis*, but the fungus has also been implicated in stock health problems (e.g. Fletcher, 1993).

It was against this background that the potential of classical biological control was recognised as an ideal complement to plant resistance; moreover, it had been noted elsewhere that there can be synergy between plant resistance and biological control (e.g. van Emden, 1982). In the late 1980s attention was therefore directed towards the South American parasitoid *M. hyperodae*. During an initial visit to South America in 1988 (Goldson *et al.*, 1990), *M. hyperodae* was far more abundant and widely distributed than was expected based on Loan and Lloyd (1974) and Lloyd's unpublished CIBC reports. The opportunity therefore presented itself to 'design' a biological control programme from the outset.

The widespread distribution of *M. hyperodae* (Goldson *et al.*, 1990) permitted the collection of seven populations from contrasting habitats in Brazil, Uruguay, Argentina and Chile. After quarantine and host range testing permission was granted to release the species (Goldson *et al.*, 1992). To date, this work has produced very promising results with levels of parasitism of over 80% recorded within three years of the species' release and clear indications of pest suppression (Goldson *et al.*, 1994).

Ecological experimentation and assessment of the importance of strains to the success of *M. hyperodae*

The various *M. hyperodae* strains and the range of New Zealand climate zones into which the parasitoid was released offered a good opportunity for ecological experimentation. Since 1991 there have been 24 releases throughout New Zealand, the majority of which comprised over 10 000 infected weevils each (Goldson & Barker, 1995). Considerable effort was made in each release to ensure that equal numbers of each strain were released at all sites. In this way it was hoped that analysis of spatial and temporal variation in the strains' establishment patterns could be made. This objective has already been achieved in part using a morphometrical approach whereby the South American east and west coast *M. hyperodae* strains can be differentiated (Phillips & Baird, 1995). From this work there is clear evidence that in the three years immediately following the species' release the east coast strains have been more successful than those from the west coast (Phillips *et al.*, 1994; Phillips, unpublished data). Work is currently being carried out on DNA-based methods of strain differentiation to take this study further. *M. hyperodae* collected from the field in New Zealand are being stored at -80°C pending the development of suitable methods.

At its most rewarding, this study should yield information on questions such as whether the same

strain established throughout New Zealand, whether the rapidly establishing strains are usurped by others later and whether some strains migrate more rapidly than others. The DNA-based techniques being developed for this study could also shed new light on the nature and genetic stability of *M. hyperodae* parthenogenecity.

Analysis of factors that pre-dispose a biological control agent to success

Many authors (e.g. Messenger & van den Bosch, 1971; Caltagirone, 1985) have indicated the importance of identifying the correct strain of a biological control agent for biological control purposes although exactly how to do this remains uncertain. Ehler (1990) summarised eight factors seen by a number of workers to be important attributes of natural enemies (e.g. fitness and adaptability, high searching capacity, sufficient power of increase etc), although it has been recognised that this approach has limitations such as the lack of coincidence of all factors within one species (Waage 1990). Roush (1990) pointed out that of all the purported factors that do contribute to the success of biological control agents, in general only their adaptability to local climate conditions and ability to avoid host defence seem to be critical.

In the *L. bonariensis* biological control programme, the seven South American strains of *M. hyperodae* have been maintained separately in laboratory culture. This has permitted laboratory-based analysis of biological variation between the *M. hyperodae* strains to be conducted concurrently with the parasitoid release programme. It is hoped that identification of each strain's key biological characteristics, combined with the experimental basis of the parasitoid release programme, will eventually allow some definition of what confers adaptive advantage to the strains in New Zealand's different climate zones. Some progress has been made in this respect already. It has been found for example that the Brazilian strain of *M. hyperodae* does not enter photoperiodically-induced diapause whereas the others do (Goldson *et al.*, 1993); such an observation could provide insight into the adaptive value of *M. hyperodae* diapause in different parts of New Zealand. Recent work has also shown that by laying a mean of 62 eggs per parasitoid, the fecundity of the Uruguayan *M. hyperodae* strain is almost twice that of the four other strains analysed to date (unpublished data).

DNA-based investigation into the geographical origin of the New Zealand *L. bonariensis* population

During collection of the different South American strains of *M. hyperodae* populations, 12 South American populations of *L. bonariensis* were also collected. These were imported live into New Zealand and preserved at -80°C with a view to developing DNA-based techniques to determine the geographical origin of the New Zealand weevil population. Using a method based on PCR-RAPDS, Williams *et al.* (1994) demonstrated that the genome of New Zealand population is very similar to that of the Australian population and that there has apparently been very little change in the genome of the New Zealand population since it was accidentally introduced. Williams *et al.* (1994) and Williams (1994) were also able to show that there is very little genetic variation in the New Zealand populations from one region to the next and there is also weaker evidence that the founder population was probably genetically quite limited. Williams *et al.* (1994) achieved their original aim by demonstrating that the New Zealand/Australian populations of *L. bonariensis* probably originated in the River Plate area near Uruguay.

The use of DNA-based methods in this way helps to answer long-asked questions. For example, the genetic uniformity of the New Zealand population means that differences in the patterns of establishment of the different *M. hyperodae* strains are not due to varying host genetics. Furthermore, knowledge of the likely area of origin of the pest species could allow the importance of *L. bonariensis*-*M. hyperodae* co-evolution to be compared with the importance of collecting *M. hyperodae* from an area with a similar climate to the intended release region. To this effect, the preliminary observations that the 'east coast' parasitoids appear to be the most successful (Phillips *et al.*, 1994) and that the Uruguayan strain has a comparatively higher fecundity, perhaps points to co-evolution rather than climate matching as a critical factor

Finally, if it is possible to define the geographical origin of a pest species, this would indicate to biological control practitioners where to look for other co-evolved control agents such as pathogens.

BIOLOGICAL CONTROL THEORY AND MODELLING

The role of theory and models in biological control have been reviewed by Waage (1990), Karieva (1990), Barlow and Goldson (1990, 1993) and Barlow (1993). In spite of the abundance of theory on parasite/host and predator/prey interactions, little use has been made of this in practical biological control, let alone in the specific area addressing the effectiveness of different strains of the same agent. This may be because theory has addressed different questions to those asked by the practitioners, or because any answers obtained are at too strategic a level to have a meaningful impact on tactics. Certainly there are difficulties in applying the simple models which underpin much of this theory to complex real-world problems (Barlow, 1993). Potentially, however, models of intermediate complexity offer a number of practical benefits (Barlow & Goldson, 1990, 1993; Barlow, 1993) which include: 1) predicting the outcome and success of a specific introduction; 2) aiding in the selection of the most appropriate agent(s) or strain(s); 3) predicting the impact of exotic agents on ecosystems and non-target species; 4) increasing understanding of the processes involved; 5) aiding in the identification and interpretation of critical field data; and 6) optimising management of existing and introduced biocontrol agents.

Modelling was used in both case studies described here. For the *S. discoideus* programme it was necessarily retrospective, but it added to the understanding of the parasitoid's success, particularly by demonstrating that *M. aethiopoides* did account for the full extent of observed pest suppression. The model also confirmed the hypothesis that observed levels of parasitism over summer could be obtained by atypical development of those parasitoids oviposited in newly-emerged adult weevils (Goldson *et al.*, 1990).

In the case of the *L. bonariensis* programme, modelling continues to be carried out concurrently with ecological analysis. Prior to the parasitoid's release, a model of its phenology based on development rate data for the different strains obtained under quarantine, showed the extent of host/parasitoid synchrony and the expected number of parasitoid generations in various regions of New Zealand. It suggested that small differences in development rates between strains were unlikely to be significant, but conversely the absence of diapause in the Brazilian strain could be disadvantageous by exposing larvae and pupae to additional winter mortality. Possibly for the first time, the model also provided advance predictions of anticipated parasitoid behaviour and impact, based on field experiments on parasitoid attack rates soon after release (Barlow *et al.*, 1993; Barlow *et al.*, 1994). However, it became apparent that knowledge of the pest was insufficient, nevertheless this had the useful consequence of guiding further ecological analysis in order to refine predictions of parasitoid impact and final steady states (if any). These predictions include the effects on pasture damage and the likely consequences of combining biological control with resistant, high-endophyte grasses, all of which will require continuing and intensive data collection. Such work will be tested against the actual outcomes in order to help understand the importance of ecotypic pre-adaptation and climate matching.

CONCLUSION

There is growing international recognition of the need to maintain biodiversity and to avoid the dangers that introduced polyphagous biological control agents may present to non-target species. This has reduced the range of suitable agents available for biological control and could be one reason for increasing interest in subspecific variation. Such variation may be used to optimise the selection of appropriate strains within a beneficial species. These developments have coincided with the development of new DNA-based methods that allow differentiation between strains.

The New Zealand work described in this contribution suggests that intraspecific variation could be important to the success of two biological control programmes based on *M. aethiopoides* and *M. hyperodae*. It is hoped that some of the approaches taken in the *M. hyperodae* programme have started to address the challenges issued by Roush (1990) and Waage (1990) who have called for a more experimental approach to biological control.

Finally, the success and nature of the impacts of *M. aethiopoides* and *M. hyperodae* indicated that common factors may be involved. In both cases the weevil species built up to enormous numbers, as often happens in the absence of naturally occurring regulatory factors. New Zealand has numerous other examples of weeds and pests (both invertebrate and vertebrate species) that have done this. With respect to the programmes discussed in this contribution, Goldson *et al.* (1994) suggested that the parasitoids were able to progress in a way very similar to their respective pest host species and presumably for

similar reasons. They contended that relatively unfilled niches have offered little constraint to rapid population growth. Finally, both parasitoid species have high searching efficiencies (Barlow & Goldson, 1993; Goldson & McNeill, 1994) and *M. hyperodae*, if not both species, originated in regions where hosts are relatively rare in their unmodified habitats. Consequently the parasitoids' host-finding abilities are probably well in excess of what is needed in New Zealand's pastoral habitat.

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