VARIATION IN THE CRITICAL PHOTOPERIOD FOR DIAPAUSE INDUCTION IN *MICROCTONUS HYPERODAE*, A PARASITOID OF ARGENTINE STEM WEEVIL

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SUMMARY

A study was conducted to examine the effect of naturally occurring photoperiod on diapause induction of the Argentine stem weevil parasitoid *Microctonus hyperodae*. Results revealed that parasitoids collected from Hilario Ascasubi (39°S, Argentina) entered diapause at a photoperiod $\leq 12.40:11.60$ L:D. This contrasted with the response of a population from Porto Alegre (30°S, Brazil) which at a photoperiod of 11.55:12.05 L:D still did not fully enter diapause. Such a latitudinal shift in critical photoperiod has significant implications for phenological modelling and analysis of ecotype establishment patterns.

Keywords: *Microctonus hyperodae*, diapause, critical photoperiod

INTRODUCTION

The braconid parasitoid *Microctonus hyperodae* was released in New Zealand in 1991 as a potential biological control agent of the Argentine stem weevil *Listronotus bonariensis*. The populations imported for quarantine/host range evaluation (Goldson *et al* 1992) and eventual release were collected from a wide range of South American ecoclimatic zones encompassing Brazil, Uruguay, Argentina and Chile (Goldson *et al* 1990). For the purposes of experimentation and behavioural analysis, the different populations have been treated as distinct ecotypes. While indeed there may be less than seven ecotypes, the reverse prospect of sibling species cannot be dismissed. Breeding experiments are precluded by the parasitoid's thelytokous parthenogenetic reproduction and consequently morphometric, allozyme and DNA-based studies are being conducted to explore further these biogeographical considerations.

Recent results have indicated that the parasitoid has established in both the North (G.M. Barker pers. comm.) and South Island (S.L. Goldson unpublished data). As generally pointed out by van den Bosch *et al* (1982), the chances for success in a biological control endeavour such as this are optimised by detailed assessment of host-natural enemy distribution, the degree of parasitoid preadaptation and seasonal parasitoid-host synchrony. Since the *M. hyperodae* population imported was probably genetically quite diverse, there is likely to be a corresponding range of behavioural characteristics. It is hoped to eventually define as many of these differences as possible and reconcile them with both transient and equilibrated patterns of ecotype establishment in New Zealand's various climate zones. In this way ecological theory on what predisposes a parasitoid to success may be advanced.

To this effect, initial investigation has already shown differences in development temperature thresholds, accumulated heat requirements for development (M.R. McNeill unpublished data) and morphology (C.B. Phillips pers. comm.). Additionally it has been generally found that *M. hyperodae* enters photoperiodically induced diapause, irrespective of the endocrinal condition of its host (Goldson *et al* in prep). Diapause is marked by arrested development at the first instar larval stage. This contribution reports on ecotypic differences in response to photoperiod. An understanding of such variation is critical to phenological modelling and could well be an important element in the differential geographical establishment of parasitoid ecotypes.

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METHOD

The parasitoids used in this study were collected from Porto Alegre, Brazil (latitude 30°S) and Hilario Ascasubi, Argentina (latitude 39°S). These localities represent a latitudinal shift of c.1100 km. Both collection sites were within 20 km of the eastern seaboard at altitudes of <150 m above sea-level. Consequently there were no strikingly different altitudinal or continental effects. Apart from the River Plate there are no major geographical barriers between these sites.

Two consecutive experiments were established whereby parasitised weevil populations were exposed for a total of 50 days to naturally occurring photoperiods. The first of these was established on 5 March 1992 and the second on 26 March. After exposure to adult parasitoids, insectary and laboratory conditions were used to provide different rates of accumulation of day-degrees (°D) above the development thresholds. The Porto Alegre parasitoids have been found to have a threshold of 10.5°C and require 227°D for full larval development compared with a threshold of 9.1°C for Hilario Ascasubi and a thermal requirement of 236°D (S.L. Goldson unpublished data). In the insectary, temperatures were recorded hourly using a data-logger thereby allowing elapsed physiological time to be calculated directly. Conversely, in the laboratory, daily maxima and minima were recorded; this necessitated estimating physiological time using the method of Barlow and Dixon (1980).

In both experiments parasitism of six populations of 100 weevils was achieved over a 11 day period under insectary conditions in six 220 mm x 130 mm x 75 mm transparent cages. Three of these cages each contained eight parasitoids from Hilario Ascasubi introduced and the remainder eight parasitoids from Porto Alegre. All cages were supplied with a bouquet of ryegrass. After inoculation subsamples of c.10-20 weevils were removed from each and dissected to determine that adequate levels of parasitism had occurred. The remaining weevils from each infection cage were then divided equally and the two subpopulations transferred to 100 mm diameter x 60 mm deep transparent holding cages (Goldson and Proffitt 1990). Three rearing cages of weevils containing each parasitoid ecotype were then transferred to the laboratory and the remainder left in the insectary. Throughout the experiment the rearing cages were supplied twice weekly with ryegrass bouquets and examined regularly for prepupal emergence. After the elapsed 50 days the surviving weevils were dissected to assess whether or not they had been parasitised and if so, whether the parasitoid larvae had progressed beyond the diapausing first instar stage.

Weevils for the first experiment were swept from ryegrass pasture (cv. Manawa) at Lincoln on 20 February 1992 and stored at 10°C 16:8 L:D until required. Weevils for the second experiment were similarly collected on 18 March 1992 and stored under the same conditions.

RESULTS

Experiment 1

The development of the Porto Alegre and Hilario Ascasubi parasitoid larval populations during Experiment 1, expressed as percentages of the total numbers of weevils (dead and alive) recovered, is summarised in Table 1. During the 11 day parasitoid exposure period, photoperiod declined from 12.78:11.22 to 12.34:11.66 L:D. At the completion of the experiment this had declined further to 10.91:13.06 L:D. Dissection showed that a 96% level of parasitism had been achieved. For the duration of the experiment Hilario Ascasubi and Porto Alegre ecotypes accumulated 370°D and 313°D above threshold respectively in the laboratory (Table 1). At the same time in the insectary, the Hilario Ascasubi and Porto Alegre ecotypes accumulated 166°D and 121°D respectively (Table 1). Since *M. hyperodae* requires c.136°D to develop beyond the first instar (Goldson *et al* in prep), it is apparent that there was ample heat accumulation in the laboratory for development of both ecotypes beyond the first instar. Similarly in the insectary there was sufficient accumulated heat for the Hilario Ascasubi ecotype to have developed and marginally enough for the Porto Alegre ecotype (Table 1). Given this adequate heat accumulation, it is significant that there was minimal development of the Hilario Ascasubi ecotype in the laboratory (1.7%)

and none at all in the insectary (0.0%) (Table 1). This contrasts markedly with the 53.1% and 30.3% development of the Porto Alegre ecotype in the laboratory and insectary respectively and demonstrates further that heat accumulation had not been limiting to *Hilario Ascasubi* development.

TABLE 1: Development of the Porto Alegre and *Hilario Ascasubi* parasitoid larval populations during Experiment 1 expressed as percentages of the total numbers of weevils (dead and alive) recovered. Experiment established on March 05

Parasitoid ecotype	Accumulated °D above threshold	Caging mortality of weevils as a % of the total	Pupae and parasitoids >1st instar as % of total weevil population	1st instar parasitoids as % of total population
Laboratory				
<i>Hilario Ascasubi</i>	370	15.1	1.7	80.5*
Porto Alegre	313	18.6	53.1**	26.5
Insectary				
<i>Hilario Ascasubi</i>	166	22.3	0.0	75.7
Porto Alegre	121	16.2	30.3*	50.9

* Denotes a significantly (P = 95%) higher value than the other ecotype

** Denotes a significantly (P = 99%) higher value than the other ecotype

Experiment 2

During the period of parasitoid exposure, photoperiod declined from 11.94:12.06 to 11.55:12.45 L:D, thereafter declining to 10.26:13.74 L:D when the experiment was completed. Dissection showed that there was a 97% level of parasitism comparable to Experiment 1. The development of the Porto Alegre and *Hilario Ascasubi* parasitoid larval populations during Experiment 2 expressed as percentages of the total numbers of weevils (dead and alive) recovered is summarised in Table 2. From Table 2 it is also apparent that insectary accumulated heat was well below the 136°D threshold needed for development beyond the first instar. Conversely there was adequate accumulated heat for laboratory population development; again, however, there was no development beyond first instar larvae in the *Hilario Ascasubi* ecotype and conversely 21.9% development in the Porto Alegre ecotype.

TABLE 2: Development of the Porto Alegre and *Hilario Ascasubi* parasitoid larval populations during Experiment 2 expressed as percentages of the total numbers of weevils (dead and alive) recovered. Experiment established on March 26

Parasitoid ecotype	Accumulated °D above threshold	Mean percent mortality of weevils	Pupae and parasitoids > 1st instar as % of total weevil population	1st instar parasitoids as % of total weevil population
Laboratory				
<i>Hilario Ascasubi</i>	292	7.7	0	83.3
Porto Alegre	239	17.8	21.9**	58.4**
Insectary				
<i>Hilario Ascasubi</i>	83	16.8	0	81.2
Porto Alegre	51	13.4	0	81.5

* Denotes a significantly (P = 95%) higher value than the other ecotype

** Denotes a significantly (P = 99%) higher value than the other ecotype

DISCUSSION

Under conditions of sufficient accumulated heat in both experiments, the lack of Hilario Ascasubi ecotype development in both experiments may be attributed to photoperiodically-induced diapause. Photoperiod has been found to act directly on the egg and/or first instar of *Microctonus vittatae* (Wylie 1980). This may be taken to be the time of peak parasitoid sensitivity to photoperiod and the same probably applies to *M. hyperodae*.

The earliest time (ie the longest photoperiod) for Hilario Ascasubi diapause onset was when the ambient photoperiod was c.12.4:11.6 L:D, on about March 15. This time of maximum sensitivity was derived from the assumption that attack occurred half-way through Experiment 1's 11-day inoculation phase and that the egg/first instar stages were affected by daylength 5 after being laid. The 12.4:11.6 L:D result conforms well to Goldson *et al's* (in prep) earlier general estimate that *M. hyperodae* critical photoperiod was 12.3:11.7 L:D. This result considered *M. hyperodae* ecotypes from all South American locations (Goldson *et al* 1990) except Porto Alegre (S.L. Goldson unpublished data).

The continued development of the Porto Alegre ecotype is a marked exception to this generalisation. Table 1 indicates that the parasitoid was probably unaffected by photoperiod during Experiment 1. However interpretation of the 21.9% development that occurred in Experiment 2 is more difficult. For example, it could indicate that the 11.55:12.05 L:D photoperiod during the population's sensitive phase was exactly critical and therefore only a proportion of the population responded. Alternatively the population could represent a transitional mix between diapausing southern and non-diapausing equatorial *M. hyperodae* populations. In general a lengthening of critical photoperiod towards the equator is a well recognised trend in many species (e.g. Tauber *et al* 1986) although this would appear to be the first indication of latitude affecting parasitoid critical photoperiod.

This result provides additional evidence for the existence of *M. hyperodae* ecotypes and implies that there has been little or no population mixing across South American ecoclimatic zones. The ecological implications of such a differential response to photoperiod may be considerable. Limited or no diapause in the Porto Alegre ecotype may be an advantage phenologically as it would permit additional generations to develop. Conversely, loss of generational synchrony and/or the appearance of susceptible parasitoid stages during the winter may reduce adaptive fitness. The net effect of these opposing influences may manifest itself differentially in New Zealand's different climate zones. Field and prospective modelling studies are continuing to examine the impact of this and other ecotypic characteristics.

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