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LETHAL TEMPERATURES OF DIAPAUSING *BATHYPLECTES CURCULIONIS* (HYMENOPTERA: ICHNEUMONIDAE) A PARASITE OF THE ALFALFA WEEVIL (COLEOPTERA: CURCULIONIDAE)[1]

Ronald H. Cherry, Edward J. Armbrust, and William G. Ruesink[2]

ABSTRACT

Seasonally acclimatized diapausing larvae of *Bathyplectes curculionis* (Thomson) were exposed to extreme high and low temperatures to determine lethal temperatures for this stage of the parasite. The possible effects of relative humidity on high temperature mortality, mortality induced by repetitive exposures to sublethal temperatures, and differential survival between sexes, were also measured.

The upper lethal temperature for summer larvae was 60°C (LD_{50} from 2 to 4 h), and the lower lethal temperature for winter larvae was -25°C (LD_{50} from 0 to ½ h). Summer larvae showed significantly increased mortality with repetitive exposures to sublethal temperatures (55°C) whereas winter larval mortality did not increase significantly with repetitive exposures to sublethal temperatures (-20°C). In winter experiments in which the sex of the emerging adult could be measured, no significant difference in survival was found between the sexes.

Our results, in conjunction with published field data, strongly suggest that heat kill in the summer may be a significant mortality factor in warmer areas of the parasite's range.

INTRODUCTION

Bathyplectes curculionis (Thomson), an important parasite of the alfalfa weevil, *Hypera postica* (Gyllenhal), was first introduced into the United States in 1911 (Chamberlin, 1924). In the Great Lakes Area, the spread of the alfalfa weevil was followed by that of the parasite. More specifically the parasite was first reported in Michigan in 1969, Wisconsin in 1968, Minnesota in 1972, and in Quebec and Ontario in 1969 (Richard Dysart, personal communique). *B. curculionis* is currently established in all 48 contiguous states. Since *curculionis* occupies such a large geographic range and diapauses during the summer, fall, and winter as larvae in cocoons (Fig. 1) found in alfalfa litter, we thought temperature extremes may be an important mortality factor on this seemingly susceptible stage of the parasite. Van den Bosch and Messenger (1973) give examples of temperature as a factor limiting the success of introduced natural enemies, and Hamlin et al. (1949), van den Bosch and Dietrich (1959), and Cherry and Armbrust (1975) have suggested the possibility of heat extremes killing *curculionis* diapausing larvae under field conditions. Our study was undertaken to examine more specifically the effect of extreme temperature on survival of diapausing *curculionis* in order to compare these results with existing field data.

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MATERIALS AND METHODS

B. curculionis cocoons were obtained from parasitized alfalfa weevil larvae collected from Mason County, central Illinois, during May and June, 1974 and 1975. Alfalfa weevil larvae were fed alfalfa and held at room temperature until *curculionis* cocoon development was completed, and subsequently the cocoons were hand collected from the litter. To insure seasonal acclimatization to temperature, cocoons were stored in pint cartoons throughout the year in an outside insectary at Urbana, central Illinois. Prior to each temperature test, viability of diapausing larvae within the cocoons were determined via "candling" (Cherry and Armbrust, 1975).

Parasite larvae in cocoons were exposed to different temperatures for different time periods in constant (±1°F) temperature cabinets. High temperature experiments were made on summer acclimatized larvae during August-September, 1974-1975 and low temperature experiments on winter acclimatized larvae during January-February, 1975-1976. After each temperature exposure, larvae were held 6-7 days at room temperatures, and then the cocoons dissected to determine larval survival.

The high lethal temperature (100% mortality) was determined by exposing 40 larvae per temperature at 5°C increments from 40°C to 80°C for 6 h, a time chosen to approximate duration to extreme temperature during one day. At each temperature, 20 larvae were held at high humidity (95±5%) and 20 larvae at low humidity (20±5%) since humidity has been shown in some insects to affect survival at high temperatures (Wigglesworth, 1972). Humidity was controlled by KOH solutions (Solomon, 1951). The low lethal temperature (100% mortality) was determined by exposing 20 cocoons per temperature at 5°C increments from 0°C to −40°C for 6 h periods, humidity being disregarded.

LD_{50} values (mean lethal dose) at temperature extremes were determined by exposing 20 larvae per time interval at ½, 1, 2, 3, 4, and 5 h intervals at the high and low lethal

Fig. 1. *B. curculionis* adult and cocoon containing diapausing larva. Cocoon length is approximately 4 mm.

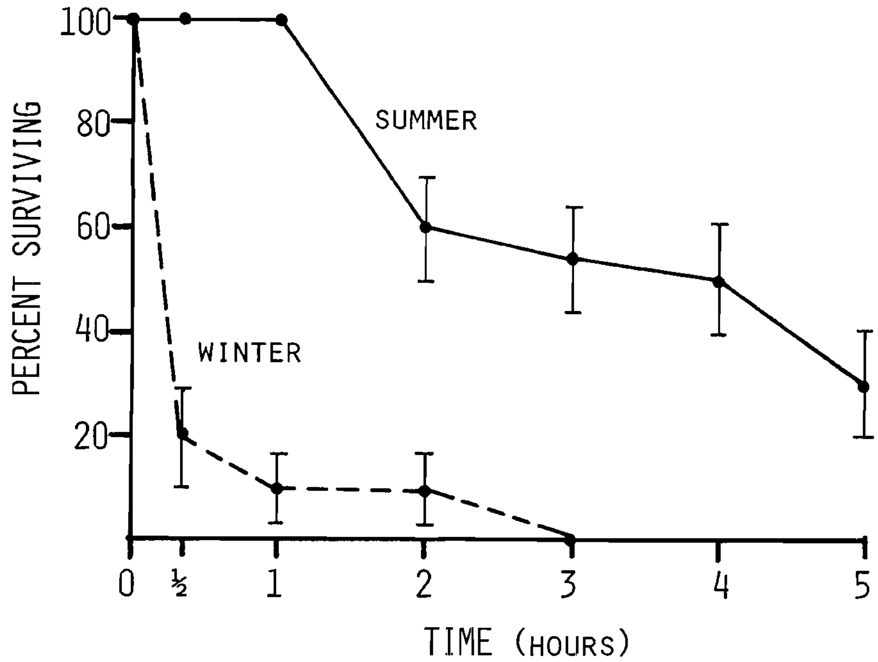

Fig. 2. *B. curculionis* survival ($\bar{x}$±SE) of summer and winter larvae at lethal temperature exposures (60°C and −25°C, respectively) for different time durations.

temperatures. Humidity at both high and low temperatures was disregarded since our previous tests showed that humidity did not affect survival at high lethal temperatures at 6 h periods.

The possibility of sublethal temperatures causing mortality via repetitive exposures was also tested. Twenty larvae were exposed to either 1, 2, 3, 4, or 5 repetitive 6 h exposures at temperatures 5°C less than the high lethal temperature, or 5°C more than the low lethal temperature. Larvae were insectary stored for 18 h between each 6 h repetitive exposure.

In order to determine the sex of the emerging adults, during the 1976 low temperature experiments, larvae which had broken diapause were allowed to complete development at room temperature. Low temperature experiments were also replicated on an additional 500 larvae. These adults versus the expected sex ratio determined from emergent adults of 100 randomly chosen cocoons were compared to determine if sex affected temperature tolerance.

RESULTS AND DISCUSSION

During a single 6 h exposure, survival of summer acclimatized larvae remained high ($\geqslant$ 85%) from 40° to 55°C, and then 100% mortality occurred at 60°C at both high and low humidities. The effect of humidity on heat death in insects appears to be highly variable, changing with insect size and time duration of temperature exposures (Wigglesworth, 1972).

Results of high temperature exposures on summer larvae are shown in Figures 2 and 3. Standard errors computed were based on the binomial distribution. Summer larvae

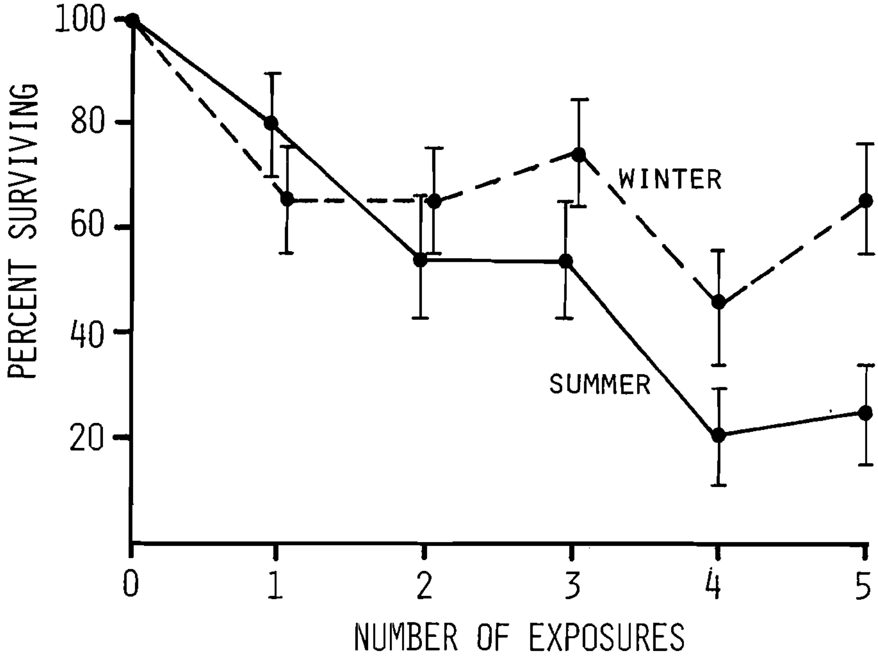

Fig. 3. *B. curculionis* survival ($\bar{x}$±SE) of summer and winter larvae exposed repetitively to sublethal temperatures (55° and –20°C, respectively). Duration of each exposure is 6 h with 18 h insectary storage between exposures.

showed gradually decreased survival with increased time exposure at the upper lethal temperature (60°C), the LD_{50} at 60°C being from 2 to 4 h (Fig. 2). There was also a significantly decreased survival of summer larvae (Fig. 3) with repetitive exposures to sublethal temperatures (55°C). For comparative purposes, it should be noted that for short exposures of about 1 h in duration, the upper lethal temperature limit for most insects lies between 40° and 50°C (Bursell, 1974), temperatures more moderate than our determined upper lethal temperature (60°C).

Survival of winter acclimatized larvae remained high ($\geqslant$ 85%) from 0 to –20°C for a single 6 h exposure, and then 100% mortality occurred at –25°C. Winter larvae showed sharply decreased survival with increased time exposure at the lower lethal temperature (–25°C), the LD_{50} at –25°C being from 0 to ½ h (Fig. 2). In contrast to summer larvae, winter larvae did not show significantly decreased survival (Fig. 3) with repetitive exposures to sublethal temperatures (–20°C). Although the mechanisms of *curculionis* cold tolerance are not shown, it is very interesting to note that in many overwintering insects, even in those which possess no glycerol or other protective substances, the supercooling point very frequently occurs in the vicinity of –20°C (Asahina, 1966).

Chi-square analysis of low temperature experiments in which 301 adult survivors were sexed showed no significant difference in survival between sexes. In contrast, greater tolerance to temperature has been noted in females of two species of *Drosophila* (Tantawy and Mallah, 1961).

Temperature induced mortality on diapausing *curculionis* larvae under field conditions has been suggested by earlier investigators. Hamlin et al. (1949) in Utah, and van den Bosch and Dietrick (1959) in California, have stated that summer heat may kill diapausing *curculionis* larvae. Cherry and Armbrust (1975), using *curculionis* larvae planted in alfalfa fields in Illinois, found that the lowest monthly survival in recovered larvae was in July, which has the highest monthly temperature in Illinois, and that the average monthly survival was significantly lower in summer than in fall or winter. Pinter et al. (1975), in freshly cut alfalfa fields in Arizona, have recorded ground surface temperatures of 63°C which exceeds our determined upper lethal temperature limit for *curculionis* larvae. Conversely, the lowest ground surface temperature recorded in alfalfa fields near Lexington, Kentucky, during 1966-67 winter months was -10°C (Pass, unpubl.), a temperature well above the lower lethal temperature of *curculionis* larvae. Hence, from the above field observations, in conjunction with our lethal temperature determinations, we conclude that *curculionis* larvae appear to be more susceptible to summer heat kill than winter cold mortality, and that summer heat may be a significant mortality factor in warmer areas of the parasite's range.

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