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## THE EFFECT OF REPEATED LOW TEMPERATURE ON EGGS OF THE ALFALFA WEEVIL (COLEOPTERA: CURCULIONIDAE)<sup>1</sup>

L. J. Crain and E. J. Armbrust<sup>2</sup>

### ABSTRACT

Three ages of alfalfa weevil, *Hypera postica* (Gyllenhal) eggs were exposed to repeated exposures of  $-15$  and  $-20^{\circ}\text{C}$ . Fresh-laid eggs were quite susceptible and 3- and 5-day old eggs were relatively resistant to  $-15^{\circ}\text{C}$ , but all ages of eggs showed considerable susceptibility to  $-20^{\circ}\text{C}$ , with an average  $\text{LT}_{50}$  of 2.2 days. Comparison of this data with similar studies utilizing constant low temperature exposures showed the effect to be independent of temporal interruptions.

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The alfalfa weevil, *Hypera postica* (Gyllenhal), is the principal pest of alfalfa in the United States. In many areas, once adults reach sexual maturity in the fall, they have the capability to oviposit throughout the winter whenever temperatures reach ca.  $10^{\circ}\text{C}$  (Hamlin et al., 1949). Fall- and winter-laid eggs may account for 50% of the spring larval populations (Evans, 1959), while the remainder of the larvae hatch from spring-laid eggs.

The importance of temperature as a mortality factor of overwintering eggs has been discussed by several workers (Drea, 1969; Hsieh and Armbrust, 1974; Miller and Shaw, 1967; Niemczyk and Flessel, 1970; Roberts et al., 1970; and Streams and Fuester, 1966), most of whom based their observations on field sampling data.

Armbrust et al. (1969) found the supercooling point of exposed eggs to be as low as  $-25.2^{\circ}\text{C}$  and Morrison and Pass (1974) tested the survival of eggs of various ages at a wide range of temperatures and constant exposure times. These researchers provided temperature mortality thresholds, but did not consider the effect of repeated exposures to low temperatures interspersed with sublethal temperatures, conditions which often occur naturally. This study was undertaken to study the effect of repeated low temperature exposures on alfalfa weevil egg mortality and to provide parameter values for simulation and predictive models of alfalfa weevil biology.

### METHODS AND MATERIALS

Eggs used in this study were obtained from fall-collected adults from Washington County, Illinois. Adults were stored at a constant  $4 \pm 2^{\circ}\text{C}$  until needed for oviposition, at which time they were held at  $15 \pm 3^{\circ}\text{C}$ ,  $30 \pm 5\%$  relative humidity with bouquets (10 stems) of fresh greenhouse alfalfa. Eggs were extracted from stems daily using a modification of the blender method developed by Pass and Van Meter (1966). For temperature testing, eggs were placed on filter paper in inverted glass petri dishes with a thin plaster of paris layer on the bottom. The plaster was saturated with a .001 aqueous solution of benzalkonium chloride for mold inhibition. The inside edge of the dish was ringed with cotton dental wicks. Both cotton wicks and filter paper were saturated with the benzalkonium chloride solution to inhibit mold during incubation. When the

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incubation dishes were tested for possible insulative properties, it was found that the interior of the dish required 5.3 minutes to cool from 22.5°C to 2.0°C and 22.6 minutes to reach the chamber temperature of 0°C. When the environmental chamber was set at -20°C, the dish required 5.1 minutes to cool to -15°C and 27.0 minutes to cool to -20°C. Thus any influence of the incubation dish on cooling data was considered inconsequential to the data.

To determine the effect of repeated exposure to low temperature, 50 eggs at three stages of development (fresh, 3-, and 5-day old) were exposed to each test temperature of -15 and -20°C for either 1, 2, 3, 4 and 5 repetitive 6 hour periods. The test temperatures of -15 and -20°C were selected in light of the mortality thresholds obtained by Morrison and Pass (1974). Eggs were stored at  $4 \pm 2^\circ\text{C}$  for 18 hours between each exposure.

### RESULTS AND DISCUSSION

The percent hatch of three ages of alfalfa weevil eggs after repeated exposures to -15 and -20°C is presented in Table 1 and a regression analysis is illustrated in Figure 1. At -15°C, fresh eggs showed a marked drop in hatch with time; the time required to kill 50% of the population (LT<sub>50</sub>) was 1.2 days. Eggs that had been aged for 3 and 5 days were relatively resistant to -15°C. Eggs of all ages showed a steady decrease in hatch at -20°C although aged eggs withstood longer exposures, as evidenced by the LT<sub>50</sub> values of 2.1, 2.7, and 4.0 days for fresh, 3- and 5-day-old eggs, respectively. Regression formulae and correlation coefficients for Figure 1 are presented in Table 2. With the exception of the equations for fresh and 3-day-old eggs exposed to -20°C, none of the  $r$

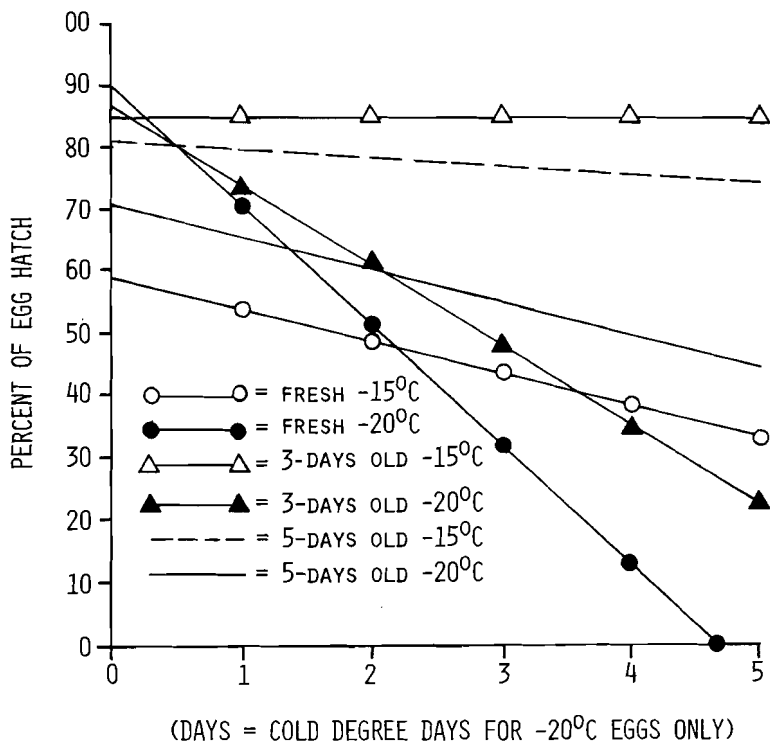


Fig. 1. Percent hatch of alfalfa weevil eggs of various ages exposed to -15 and -20°C.

values were significant at the .05 level. For this reason, the data for exposures showing no decrease in hatch with time (3- and 5-day at  $-15^{\circ}\text{C}$ ) were lumped as were those exposures showing a decrease in hatch ( $15^{\circ}\text{C}$  fresh eggs and all ages at  $-20^{\circ}\text{C}$ ). The resulting formulae are found at the bottom of Table 2 and the regression lines in Figure 2. After lumping, the 3- and 5-day-old eggs showed no significant correlation at  $-15^{\circ}\text{C}$  as

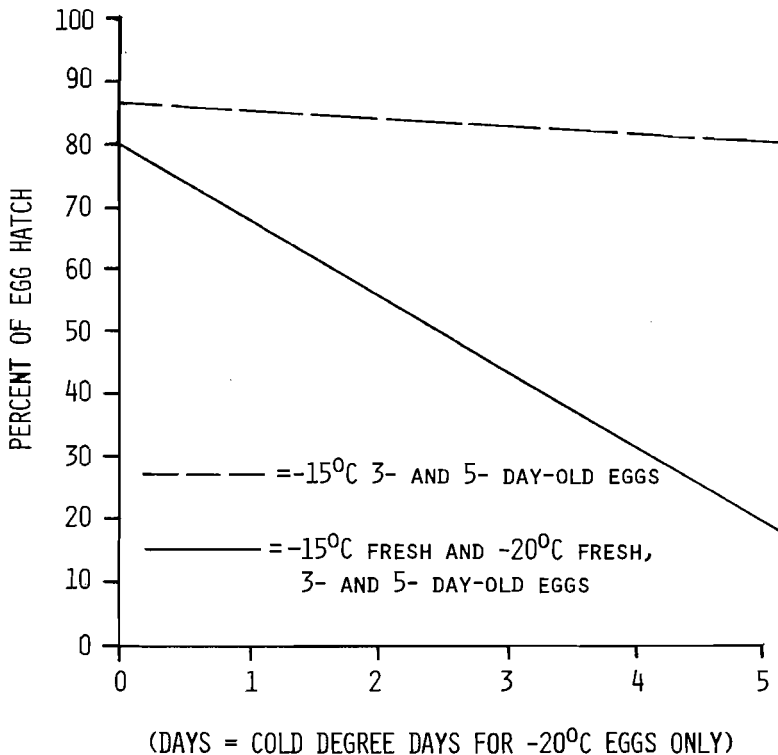


Fig. 2. Percent hatch of 3- and 5-day old alfalfa weevil eggs exposed to  $-15^{\circ}\text{C}$  and fresh and 3- and 5-day old eggs exposed to  $-20^{\circ}\text{C}$ .

Table 1. Percent hatch of three different ages of alfalfa weevil eggs after repeated exposure to  $-15$  and  $-20^{\circ}\text{C}$ .

| Number of repeated exposures <sup>a</sup> | Age of eggs (days) and exposure temperature ( $^{\circ}\text{C}$ ) |     |      |     |     |     |
|---|--|-----|------|-----|-----|-----|
|   | 0-1  |     | 3    |     | 5   |     |
|   | -15  | -20 | -15  | -20 | -15 | -20 |
| 0   | 79   | 90  | 84.0 | 92  | 82  | 84  |
| 1   | 44   | 69  | 81.0 | 80  | 73  | 30  |
| 2   | 33   | 70  | 89   | 32  | 80  | 84  |
| 3   | 53   | 10  | 77   | 40  | 80  | 20  |
| 4   | 62   | 8   | 82   | 66  | 71  | 36  |
| 5   | 25   | 6   | 85   | 8   | 74  | 22  |

<sup>a</sup>One exposure consisted of 6 hours at  $-15$  or  $-20^{\circ}$  and the remaining 18 hours at  $4 \pm 2^{\circ}\text{C}$ .

Table 2. Alfalfa weevil egg mortality regression equations.

| Temperature (°C) | Age (days)   | Equation             |                     |
|------------------|--------------|----------------------|---------------------|
| -15              | Fresh        | $y = .5776 - .0497x$ | -.3613              |
|                  | 3            | $y = .8332 - .0001x$ | -.4245              |
|                  | 5            | $y = .8021 - .0132x$ | -.5793              |
| -20              | Fresh        | $y = .8964 - .1895x$ | -.9280 <sup>a</sup> |
|                  | 3            | $y = .8543 - .1297x$ | -.7633 <sup>a</sup> |
|                  | 5            | $y = .7139 - .0529x$ | -.6306              |
| -15 <sup>b</sup> | 3 & 5        | $y = .8176 - .0066x$ | -.2272              |
| -15 <sup>c</sup> | Fresh        |                      |                     |
| -20 <sup>c</sup> | Fresh, 3 & 5 | $y = .7610 - .1174x$ | -.6861 <sup>a</sup> |

<sup>a</sup>Significant at .05 level.

<sup>b</sup>Combined mortality values for 3- and 5-day-old eggs at -15°C.

<sup>c</sup>Combined mortality values for fresh at -15°C with fresh 3- and 5-day-old eggs at -20°C.

would be expected with such a low slope value. All the remaining values combined showed significant correlation at .05 and an  $LT_{50}$  of 2.2 days. If this  $LT_{50}$  value is converted into hours of constant exposure it becomes 13.2 hours. Treating data presented by Morrison and Pass (1974) in a similar manner resulted in an  $LT_{50}$  of 12.8 hours for eggs of comparable age exposed to -15 and -20°C for constant periods. This  $LT_{50}$  was 97% of the value obtained in the repeat exposure study.

The results of the present study are quite comparable with those of Morrison and Pass (1974) and it appears that the effect of low temperature on alfalfa weevil egg mortality is an additive phenomenon; i.e., the effect of cold on egg mortality with time is independent of any temporal interruption during the low temperature exposure. This finding suggests that if the rate of accumulation of low temperatures in nature could be determined, laboratory studies involving constant temperature exposures could be designed to simulate natural conditions more efficiently.

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