Effects of Dormancy and Photoperiod on Alfalfa Weevil (Coleoptera: Curculionidae) Reproductive Diapause[1]

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ABSTRACT Alfalfa weevil, *Hypera postica* (Gyllenhal), adults reared as larvae in the laboratory under an 8-h photophase were subjected to three storage conditions and two photoperiods to determine their effects on female reproductive diapause and fecundity. Contrary to previous reports, weevils reared in the laboratory as larvae and adults under a short-day photoperiod remained in a partial reproductive diapause. Newly-emerged weevil adults subjected to a 5-wk dormant period followed by an 8-h photophase had a rate of oviposition nearly twice that of adults reared under an 8-h photophase without a dormant period. Adults reared under a 15-h photophase after the dormant period also remained in a partial reproductive diapause. Results demonstrate that alfalfa weevil reproductive diapause is influenced by dormancy (an inactive state) and photoperiod. This is the first report of an efficient method to break reproductive diapause in the alfalfa weevil which will enable continuous production of nondiapausing laboratory-reared weevils for research and mass production of predators and parasites for biological control.

KEY WORDS *Hypera postica,* mass-rearing, diapause, photoperiod, dormancy

In the Eastern United States, alfalfa weevil, *Hypera postica* (Gyllenhal), females lay their eggs in late fall and early spring, larvae develop and pupate in the spring, adults emerge, migrate from alfalfa fields to pass the summer in an inactive state (aestivation), and return in the fall to begin sexual development (Manglitz and App 1957, Blickenstaff et al. 1972). Based on the development of sex organs in natural field populations of females, the alfalfa weevil undergoes a true ovarian diapause (Guerra and Bishop 1962, Tombes 1964). In these studies, sexual development occurred from mid-October to mid-December and in April, the reproductive organs of new generation adults were undeveloped and remained so as adults entered aestivation.

Laboratory studies on the photoperiod-diapause relationship in the alfalfa weevil demonstrated that alfalfa weevils reared under increasing photophases have increasing times from adult emergence to first oviposition (Huggans and Blickenstaff 1964, Rosenthal and Koehler 1968, Bland 1971). These studies suggest that adult diapause is a response to the photoperiod under which larvae are reared. However, observations were made only on the preoviposition period, and no information on the rate or length of the oviposition period was presented.

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The alfalfa weevil, despite an effective biological control program (Kingsley et al. 1993), remains a major pest of alfalfa (Lamp et al. 1991, Dowdy et al. 1992), and efforts to develop alternate methods of control continue (Ratcliffe and Elgin 1990, Bernays and Cornelius 1992, Thomas et al. 1992). To support current and future laboratory screening studies to identify alternate methods of control, a continuous supply of nondiapausing alfalfa weevils is needed, particularly if adult feeding and oviposition are activities to be evaluated.

Methods currently used to supply alfalfa weevils for laboratory studies are cold storage of nondiapausing field-collected adults (Byrne 1965), which can be used as a source of eggs for succeeding generations, and cold storage of eggs for extended periods of time (Cothran and Gyrisco 1966). Hydroprene, a compound with juvenile hormone activity, has been topically applied to female alfalfa weevil adults to terminate sexual diapause but with mixed results (Ascerno et al. 1981).

Studies on the photoperiod-diapause relationship previously discussed indicate that laboratory nondiapausing alfalfa weevil colonies can be produced by photoperiod control. To date, this theory has not been documented in the literature. Unpublished preliminary studies in this laboratory have demonstrated a wide range in fecundity of alfalfa weevils reared in the laboratory under a constant 8-h photophase. These observations suggest that the dormant or inactive period field adults undergo must be part of the diapausing-breaking process along with short-day photoperiods. The objective of the present study was to determine the effects of storage (an inactive period) and photoperiod on the reproductive diapause of female laboratory-reared alfalfa weevils.

Materials and Methods

Alfalfa weevils used in this study were first generation laboratory-reared, derived from eggs from nondiapausing fall field-collected adults which had been held in cold storage at 5°C on 2% sugar water until needed (Byrne 1965). Larvae and pupae were reared on flats of greenhouse-grown 'Saranac AR' alfalfa in a walk-in environmental growth chamber maintained under a photoperiod of 8:16 (L:D) h at 24 ± 1°C and 50-90% RH (Ratcliffe and Elgin 1987). Newly-emerged adults were transferred to new flats of alfalfa and fed for 8 d prior to the beginning of the test.

After 8 d, adults were submitted to three storage conditions: None (constant alfalfa), 24°C for 5 wk on 2% sugar water, and 5°C for 5 wk on 2% sugar water. Storage on sugar water as described by Byrne (1965) was in complete darkness. Adults that were not stored were confined on alfalfa immediately and placed under either a short 8:16 (L:D) h (treatment 1) or long 15:9 (L:D) h (treatment 2) photoperiod. After 5 wk those adults in storage on sugar water at two different temperatures also were placed under either a short or long day photoperiod (treatments 3 to 6). Two photoperiods and three storage conditions gave a total of six treatments (Table 1). Treatments were maintained under their respective photoperiods in environmental growth chambers at 24 ± 1°C, 45-90% RH, and 1300 foot candles (14 klx) of light. Each treatment was replicated four times with five adults of each sex placed on bouquets of greenhouse-grown alfalfa in glass jars with ventilated lids (Ratcliffe and Elgin 1987). Alfalfa in each treatment was changed, stems split, and eggs recorded every 3 to 4 d. Also, at each change, dead

Table 1. Mean alfalfa weevil fecundity and egg viability as influenced by adult storage and photoperiod.

	Treatment*						
	Storage		Photophase	n	Eggs / ♀ / day	Eggs / ♀ / study	% Egg viability
1	NS	-	8H	152	11.35 b**	1725	85.4
2	NS	-	15H	44	0.03	2	55.4
3	24°C	-	8H	120	19.97 a	2396	86.3
4	24°C	-	15H	120	9.12 bc	1094	78.7
5	5°C	-	8H	120	21.45 a	2574	84.2
6	5°C	-	15H	120	5.03 c	604	69.5

* Storage: NS (No-storage) or 5 wk on 2% sugar H_2O at 24° or 5°C. Photophase = hours of light.

** Mean of 4 replications times number of dates. Values with different letters are significantly different, Fisher's LSD with $\alpha = 0.05$. Treatment 2 not included in statistical analysis.

adults were replaced, leaf feeding was estimated by scoring the amount of foliage consumed on a 1-5 scale (1 = none, 3 = light, 5 = heavy), and approximately 100 eggs from each treatment (if available) were incubated to determine viability. A stock jar of 200 adults (mixed sex) was maintained for each treatment to provide replacements for those insects that died in test.

Fecundity and feeding responses were analyzed as a split plot with treatment as the whole plot factor and age as the subplot factor using the SAS GLM procedure (SAS Institute 1988). Pairwise comparisons among the main-effect means for age were tested. In addition, the maximum eggs per female per day value and the age at which this maximum value occurred were estimated for each treatment using a quadratic regression analysis.

Results and Discussion

Laboratory rearing of neonate alfalfa weevil larvae to adults on flats of alfalfa under an 8-h photophase and temperature of 24 ± 1°C took approximately 21 d. Time from adult emergence to first eggs, the pre-oviposition period, for the two treatments with no storage regardless of photoperiod was approximately 26 d, similar to that reported by Rosenthal and Koehler (1968) under similar conditions. Time from adult emergence to first eggs for the four treatments in storage for 5 wk regardless of storage temperature or subsequent adult photoperiod was approximately 54 d. Oviposition occurred 11 d after adults were removed from storage for all four treatments.

All treatments except the no-storage 15-h photophase (treatment 2) were terminated when adults were 155 d old, at which time feeding and oviposition had virtually stopped. The no-storage 15-h photophase treatment was terminated when adults were 61 d old because all females remained in reproductive diapause. Adults in the stock jar for this treatment were checked weekly until 182 d old, at which time they were still in diapause. Data from this treatment were not included in the statistical analyses.

The effects of storage and photoperiod on alfalfa weevil fecundity and egg viability are presented in Table 1. The mean number of eggs per female per day in both the 24°C storage 8-h and 5°C storage 8-h treatments (3 and 5) were significantly ($P < 0.001$) greater (over 75%) than in the no-storage 8-h treatment (1). The mean number of eggs per female per day in both the 24°C storage 8-h and 5°C storage 8-h treatments were also significantly greater (over 100%) than in the 24°C storage 15-h and 5°C storage 15-h treatments (4 and 6), which were less than in the no-storage 8-h treatment. The mean number of eggs per female per day for the no-storage 8-h treatment (11.4) after 155 d was identical to that reported by Drea (1969) after 130 d under similar conditions. Egg viability was similar for all 8-h treatments regardless of storage conditions. Egg viability was higher in 8-h treatments than in 15-h treatments. These data demonstrate that both adult storage and photoperiod have a significant effect on alfalfa weevil fecundity and that photoperiod influences egg viability.

Data for alfalfa weevil adult foliar feeding, age at which feeding declined, and mortality, as influenced by adult storage and photoperiod, are presented in Table 2. Adult foliar feeding and age at which feeding declined were similar for all 8-h treatments regardless of storage conditions, taking into consideration that those

Table 2. Mean alfalfa weevil adult feeding, age at which feeding declined, and adult mortality as influenced by adult storage and photoperiod.

	Treatment*						
	Storage		Photophase	n	Feeding**	Age (days)	% Mortality†
1	NS	-	8H	152	3.2 b	106	70
2	NS	-	15H	44	1.8	29	0
3	24°C	-	8H	120	3.4 ab	134	83
4	24°C	-	15H	120	2.1 c	61	29
5	5°C	-	8H	120	3.5 a	145	50
6	5°C	-	15H	120	1.7 d	61	4

* Storage: NS (No-storage) or 5 wk on 2% sugar H_20 at 24° or 5°C. Photophase = hours of light.

** Mean of 4 replications times number of dates. Feeding scored on a 1-5 scale (1 = none, 2 = some, 3 = light, 4 = moderate, and 5 = heavy). Age is age of adults when feeding dropped below 3 (light). Values with different letters are significantly different, Fisher's LSD with α = 0.05. Treatment 2 not included in statistical analysis.

† Mortality is the % adults that died and were replaced in each treatment.

adults in the no-storage 8-h treatment fed for 35 d while adults in the other treatments were in storage. Adult feeding and age at which feeding declined were greater in 8-h treatments than 15-h treatments. Based on residuals across treatments, adult feeding was positively correlated with eggs per female per day (r = 0.34, $P < 0.001$). The number of dead adults replaced, or percent mortality, was a direct reflection of the physiological condition of the adults imposed by specific treatments. Adults exposed to conditions that resulted in higher fecundity and increased feeding (treatments 3 and 5) had the highest mortality due to their higher rate of metabolism. These data demonstrate that the photoperiod under which adults are held after emergence influences termination of diapause, which in turn, results in sexual maturation and increased feeding by adults.

Mean predicted values for alfalfa weevil fecundity, maximum oviposition rate, and age of adults at which maximum oviposition occurs, as influenced by adult storage and photoperiod, are presented in Table 3 and Fig. 1. To provide uniformity, only data at adult ages 54 to 155 were used in this analysis. The no-storage 15-h treatment, in which adults remained in diapause, was not included in this analysis. As expected, mean predicted eggs per female per day values were similar to the average values in Table 1. The maximum mean number of eggs per female per day was 32.3 at 101 d of the age for adults in the 5°C storage 8-h treatment (5) which was similar to the value of 28.7 eggs per female per day at 96 d of age for adults in the 24°C storage 8-h treatment (3). Interestingly, the adult age at maximum fecundity in the 5°C storage treatments was similar, regardless of

Table 3. Mean predicted values for alfalfa weevil fecundity, maximum fecundity, and age of adults at which maximum oviposition will occur as influenced by adult storage and photoperiod.*

	Treatment*					
	Storage		Photophase	Eggs / ♀/ day	Max eggs / ♀ / day	Age (days)
1	NS	-	8H	11.7	15.5	79
3	24°C	-	8H	20.5	28.7	96
4	24°C	-	15H	9.2	14.1	46
5	5°C	-	8H	22.2	32.3	101
6	5°C	-	15H	5.2	7.2	101

* Predicted values from quadratic regression analysis based on 4 replications and 30 dates (n = 120), ages 54 to 155.
** Storage: NS (No-storage) or 5 wk on 2% sugar H_20 at 24° or 5°C. Photophase = hours of light.

photoperiod, even though most females in the 5°C storage 15-h photoperiod treatment (6) were in reproductive diapause.

In Maryland, new adults that emerge in the spring aestivate at minimum 4 mo before they return to the field in the fall to begin sexual development. In the current study, the 5 wk storage period used to mimic aestivation was based on a preliminary (unpublished) study conducted by this author which demonstrated that female alfalfa weevils held in cold storage at 5°C for up to 5 mo laid more eggs after 1 or 2 mo in storage compared to 3 to 5 mo in storage. Oviposition in the preliminary study was measured for 57 d under a 12-h photophase.

Results of this study demonstrate that alfalfa weevil adults reared from larvae in the laboratory under an 8-h photophase and placed in storage on 2% sugar water for 5 wk at 5° or 24°C in the dark, followed by transfer to an 8-h photophase, have a rate of oviposition nearly twice that of weevils reared as larvae and adults under an 8-h photophase but without a storage or a dormant period. Average values of 22 eggs per female per day and 2574 total eggs per female for the 5°C 8-h photo-phase (Table 1) far exceeded previously reported values even for nondiapausing field-collected adults. This is the first report of a laboratory procedure, based on dormancy and photoperiod, to break reproductive diapause in laboratory-reared colonies of alfalfa weevil. This method enables continuous production of nondiapausing laboratory-reared alfalfa weevil adults for research and provides flexibility and efficiency in mass production for rearing biological control agents or use in field studies.

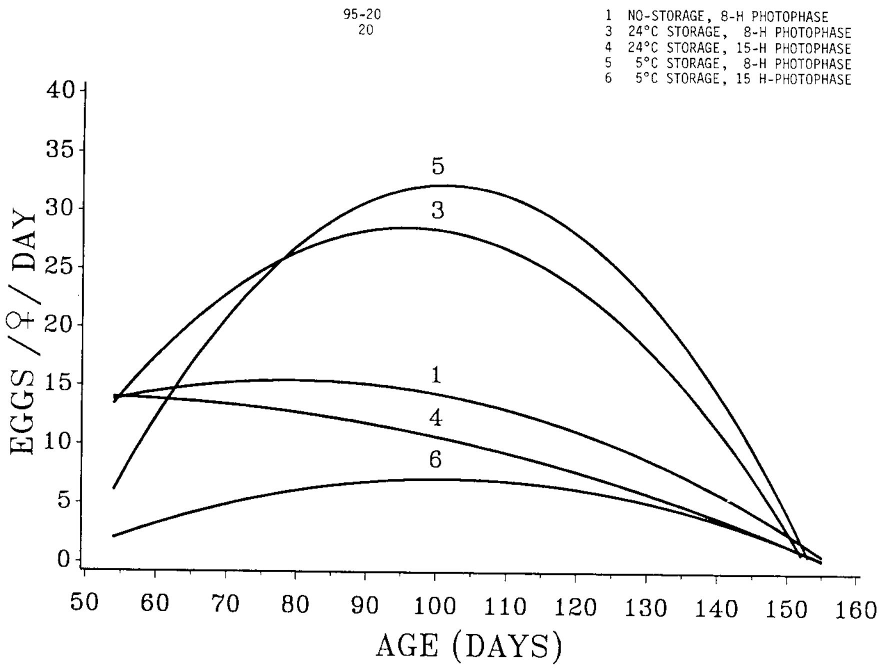

Fig. 1. Mean predicted maximum fecundity and age of adults at which maximum oviposition occurs for female alfalfa weevils reared in the laboratory.

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