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ABSTRACT

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Cold acclimation and cold tolerance were studied in laboratory-reared diapausing pink bollworms, *Pectinophora gossypiella* (Saunders), conditioned at 5°, 10°, and 15° C for 2, 4, and 8 wk. Larval mortality, abnormal pupation and total mortality from 2-h exposure to sub-zero temperatures increased similarly as the temperature decreased from −6° to −15° C. Exposure to −9° C and above had little effect.

The effect of cold conditioning on cold tolerance was most important at the −12° C exposure. Conditioning at 5°, 10°, and 15° C reduced total mortality after exposure to −12° C, compared to unconditioned larvae. Conditioning of larvae at 10° C for 8 wk resulted in the least mortality of all treatments.

Water content decreased as duration of conditioning increased, with no significant differences between conditioning temperatures. Decreased water content did not correlate well with observed changes in cold tolerance.

Temperature has been found to be an important factor affecting overwinter survival of the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders). Chapman et al. (1960) reported that winter survival of PBW larvae at El Paso, TX, was directly related to the intensity of winter cold. Survival was greatly reduced when winters had avg minimum temperatures of −2° C and below, with extremes of −19° C and below. Also, larvae held at 3.9° C and 85% RH had mortalities of 34% after 15 days, 98% after 45 days, and 100% after 60 days.

Fife and Graham (1966) studied PBW survival in bioclimatic chambers programmed for winter conditions of Heavener, OK and Waco, TX, areas of low and high winter survival, respectively. Larvae in unburied bolls held under the Heavener conditions (avg minimum, 3.6° C, avg maximum, 9.2° C and mean, 6.4° C) had less than 1/10 the survival of larvae held under Waco conditions (avg minimum, 7.1° C, avg maximum, 15.6° C and mean, 11.3° C).

Graham (1972) reported that exposure to −12.2° C for 3 or more h greatly decreased survival of disapausing PBW larvae. Decreased survival was from both larval mortality and abnormal pupation. Conditioning at 9° C for one mo increased survival after exposure to −12.2° C for one h, in terms of both larval mortality and abnormal pupation, compared to unconditioned larvae, although conditioning for one mo at 22°–27° C reduced survival.

The purpose of this study was to determine the effect of preconditioning at cool temperatures on the cold tolerance of laboratory-reared diapausing PBW larvae.

Materials and Methods

Pink bollworm eggs obtained from a culture maintained at the Cotton Insects Biological Control Laboratory, ARS, USDA, 2000 E. Allen Rd., Tucson, AZ, were surface sterilized with 0.2% (W/V) sodium hypochlorite solution and incubated at 28° C. First-stage larvae were transferred to shredded wheat germ-casein-agar diet modified from Adkisson et al. (1960), and reared in an Environator® bioclimatic chamber at 21° C and 11:13 (L:D) photoperiod. Larvae were held for an additional 3 wk under the rearing conditions after the 1st pupa was seen, to ensure that larvae were in diapause. Then, larvae were washed from the diet, sexed and held in plastic petri dishes containing paper towelling circles.

Three commercial refrigerators (0.308 m^3) modified by the addition of a heating coil, fan, thermostat and fluorescent lighting were employed for cold conditioning of larvae. The conditioning temperatures used were 5°, 10°, and 15° C. All cabinets were maintained at an 11:13 (L:D) photoperiod, with RH at ca. 30%. The cabinets set at 10° and 15° C maintained a temperature ±1° C of desired setting, with the 5° C cabinet somewhat more variable.

A sample of several hundred larvae of each sex from each conditioning group was counted weekly and larval mortality and pupation recorded. Groups of larvae (usually 25–50 of each sex) were exposed to sub-zero temperatures before and after 2, 4, and 8 wk of conditioning at 5°, 10°, and 15° C. Before and after completion of the cold conditioning, wet and dry weights of larvae (25/sex/replicate) were determined. Diapausing larvae remaining after completion of the desired cold conditioning period were divided equally into 5 groups (usually 25–50/group) and exposed to 21° C (control), −6°, −9°, −12°, and −15° C for 2 h. Sub-zero exposures were made in a freezer cabinet with circulating air capable of maintaining temperatures below zero. Larvae were exposed in plastic petri dishes with the tops removed to ensure penetration of cold.

After sub-zero exposures, larvae were held in a room at 28° C, 14:10 (L:D) photoperiod, with contact moisture. Daily counts were made thereafter to determine survival to the pupal stage.

Values of variables measured in sub-zero exposure tests were analyzed by multivariate analysis of variance. Data were expressed as decimal fractions and transformed using the angular transformation. Means were separated by Student-Newman-Keul's multiple range tests with correction for unequal replications (Steel and Torrie 1960, p. 114).

[1] Lepidoptera: Gelechiidae.

[2] Mention of a commercial product in this paper does not constitute an endorsement of this product by the USDA.

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Data for larvae conditioned 8 wk at 5° C is not reported here because of insufficient replications. Some replicates under this condition were lost because the temperature in the 5° C cabinet dropped to the lethal range.

Results

The effect of sub-zero exposure for 2 h was slight at −9° C and above in all variables measured. Exposure to −12° C and below significantly increased the larval mortality, abnormal pupation and total mortality (Table 1). There were no significant differences in the mortalities of the 2 sexes from the sub-zero exposures (F test, 0.05 level).

Table 1.—Effect of a 2-h sub-zero exposure on laboratory-reared diapausing PBW larvae (insects held at 28° C after exposure).

Exposure temperature (°C)	% larval mortality[a]	% abnormal pupation[a]	% total mortality[a]
21 (control)	16.5a	19.8a[b]	31.6a
−6	20.1a	21.0a	36.0ab
−9	23.2b	22.0a	38.9b
−12	54.6c	71.2b	83.9c
−15	80.1d	89.7c	95.5d

[a] Values are from combined data for both sexes and all conditioning treatments except 8 wk at 5° C (0, 2, 4, and 8 wk at 5°, 10°, and 15° C). All means based on ca. 100 replications. Means within each column not followed by the same letter are significantly different at the 0.05 level according to Student-Newman-Keul's multiple range test.
[b] Abnormal pupation of unconditioned larvae in 21° C control exposure= 12.2%.

The effect of cold conditioning on cold tolerance was most apparent at the −12° C exposure. Conditioning at 5°, 10°, and 15° C reduced total mortality after exposure to −12° C, compared to unconditioned larvae. Conditioning of larvae at 10° C for 8 wk resulted in the least total mortality of all treatments (Table 2). Similar effects of cold conditioning can be seen in abnormal pupation after sub-zero exposure. Larval mortality after sub-zero exposure was not affected by cold conditioning except after 8 wk conditioning at 10° C. Thus, the main effect of cold conditioning was to decrease the amount of abnormal pupation.

Larval mortality during cold conditioning increased as conditioning temperature decreased (Table 3). At all 3 conditioning temperatures little mortality occurred until after 3–4 wk of conditioning. Mortality of male larvae tended to be higher at all conditioning temperatures. Pupation was appreciable only at 15° C (Table 3) and similar rates occurred in both sexes at each temperature.

Table 2.—Effect of cold conditioning treatment on mortality of laboratory-reared diapausing PBW larvae exposed to −12° C for 2-h duration (insects held at 28° C after exposure).

Conditioning treatment ° C	Weeks	% larval mortality[a]	% abnormal pupation[a]	% total mortality[a]
Unconditioned		57.7a (18)	99.1a (18)	99.8a (18)
5	2	53.9a (10)	50.4bc (10)	73.2b (10)
	4	57.1a (10)	57.4bc (10)	81.1b (10)
10	2	56.3a (10)	72.1b (10)	84.2b (10)
	4	34.8ab (8)	61.5b (8)	75.2b (8)
	8	28.5b (8)	27.6c (8)	46.9c (8)
15	2	63.4a (12)	79.9b (12)	91.9b (12)
	4	60.2a (10)	80.4b (10)	89.5b (10)
	8	65.8a (10)	77.7b (10)	89.9b (10)

[a] Means are from combined data for both sexes. Values in parenthesis are the number of replications for the corresponding mean. Means within a column not followed by the same letter are significantly different at the 0.05 level according to Student-Newman-Keul's multiple range test.

Water content of larvae significantly decreased as duration of conditioning increased. Unconditioned larvae had 68.5% water and those conditioned for 8 wk had 63.3% water. There was no significant difference in water content between the sexes or between the conditioning temperatures at any duration (F test, 0.05 level).

Discussion

Salt (1961) discussed 2 processes that may occur during mild chilling: acclimation and chilling injury. He stated that "the cold eventually becomes injurious yet the acclimation is beneficial and probably offsets the injury to some extent. . . . Chilling injury, in contrast, accumulates in a positive manner, but as long as it is slight, acclimation remains of potential value to the insect."

In this study, cold tolerance was increased most by conditioning at 10° C for 8 wk (Table 2). The conditioning treatment at 5° C probably caused chilling injury. After 2 wk conditioning at 5° C, total mortality after exposure to −12° C for 2 h was less than that of unconditioned larvae, but after 4 wk conditioning at 5° C, cold tolerance was not increased and tended to decrease. Also, larval mortality during conditioning was greatest at 5° C.

Conditioning at 15° C for 2 wk decreased total mortality after −12° C exposure, compared to unconditioned larvae, but there was no further increase in cold tolerance as conditioning continued. This may be due to the higher temperature, since it is unlikely that chilling injury would be greater at 15° than at 10° C.

Table 3.—Effect of cold conditioning at 3 constant temperatures on laboratory-reared diapausing PBW larvae.

Age (wk)	Cumulative % larval mortality and % larvae pupating at indicated temperature (°C)[a]					
	5		10		15	
	% larval mortality	% larvae pupating	% larval mortality	% larvae pupating	% larval mortality	% larvae pupating
2	0.4	0.1	0.6	0.3	1.0	1.4
4	2.7	.2	1.9	.3	1.8	2.0
8	12.7	.2	9.6	.3	6.2	2.7

[a] Values are from combined data for both sexes.

These results are comparable to those of Graham (1972), who reported that conditioning at 9° C for one mo reduced larval mortality and abnormal pupation in laboratory-reared diapausing PBW larvae exposed to −12.2° C for one h, compared to unconditioned larvae exposed similarly. He also stated that conditioning at 22°–27° C for one mo increased larval mortality and abnormal pupation, compared to unconditioned larvae exposed as above.

Conditioning at 10° C for 8 wk resulted in the greatest increase in cold tolerance of all treatments (Table 2). Chilling injury, as suggested by the increased mortality during conditioning compared to larvae at 15° C, appears to be less important than increased acclimation in determining total mortality in this treatment. However, chilling injury may become more important after longer durations of conditioning at 10° C.

Loss of water is often temporally associated with increasing cold tolerance in insects; however, extreme dehydration may decrease cold tolerance (Salt 1961). Decreasing water content observed during cold conditioning does not appear to explain changes in cold tolerance in this study. There were no differences in water content between any of the conditioning temperatures, yet at 5° C, total mortality after −12° C exposure for 2 h tended to increase as duration of conditioning increased, while at 10° C, total mortality significantly decreased as duration of cold conditioning increased.

Salt (1961) and Asashina (1969) reported that sub-lethal effects of cold exposure often include the appearance of morphological abnormalities in the next stage. This effect can be seen in the PBW. Exposure to −12° C and below for 2 h significantly increased abnormal pupation (Table 1). However, there was a high level of abnormal pupation in the 21° C control group (Table 1). The abnormal pupae formed resemble those reported by Cawich et al. (1974) resulting from topical application of a juvenile hormone (JH) mimic on diapausing and non-diapausing PBW larvae. Wigglesworth (1952) reported that low temperatures may shift the hormonal balance in insects slightly in favor of JH. Raina and Bell (1976) reported that a hybrid strain of diapausing PBW larvae produced a low level of larval-pupal intermediates when reared at a constant 18° C, with no abnormalities produced when reared at a constant 20° or 25° C. Graham (1972) reported that laboratory-reared diapausing PBW larvae reared at a constant 29° C produced no abnormal pupae in the absence of sub-zero cold exposure. The high level of abnormal pupation seen in the 21° C control group (Table 1) might be due to low temperature disruption of hormonal balance in the laboratory strain of PBW used in this study when reared at a constant 21° C.

The amount of larval mortality and pupation during cold conditioning (Table 3) must be added onto the mortality after −12° C exposure (Table 2) in order to determine the total effect of conditioning on survival. Thus, the reported mortality of larvae conditioned at 10° C for 8 wk and exposed to −12° C is actually somewhat higher due to larval mortality (9.6%) during cold conditioning. Similarly, mortality of larvae in the other treatments is actually higher due to larval mortality and pupation during cold conditioning (Table 3). Pupation is considered as mortality because stages other than the overwintering stage often are much less cold tolerant (Salt 1961) and any pupae surviving the winter would likely emerge before suitable host plants were available for oviposition.

Salt (1961) stated that a rapid drop in temperature will result in a lower freezing point than a slower drop. In this study, larvae were subjected to a sudden large drop in temperature, from room temperature to the sub-zero exposure temperature. Compared to a field situation where the temperature drop would be more gradual, the larval mortalities reported here after sub-zero exposure may be low.

Hanec and Beck (1960) found that sub-zero cold exposure in the presence of contact moisture increased the mortality of diapausing European corn borer larvae, *Ostrinia nubilalis* (Hübner), compared to exposure to the same temperature in the absence of contact moisture. Field studies of overwinter mortality of PBW larvae have shown that higher soil moisture levels in the winter increased mortality compared to lower soil moisture levels (Chapman et al. 1960). Part of this effect was due to soil compaction and part may have been due to an effect similar to that reported by Hanec and Beck (1960). Since the sub-zero exposures in this study were done in the absence of contact moisture, the larval mortalities reported here may be low compared to a field situation with moderate soil moisture levels.

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