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TEMPERATURE DEPENDENCE–INDEPENDENCE OF ANTIFREEZE TURNOVER IN *EUROSTA SOLIDAGINIS* (FITCH)

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Abstract—Temperature effects on antifreeze metabolism were investigated in two populations (northern and southern) of the golden rod gallfly, *Eurosta solidaginis*. Sorbitol production was temperature dependent and was triggered by short-term exposure to $< +10$°C. The maximal rate sorbitol synthesis occurred at 0°C. For both populations, sorbitol was rapidly catabolized during warm acclimation at +20°C. During the first 12 h of warm acclimation, sorbitol levels decreased by 36% (19.7 ± 0.6 to 12.6 ± 1.2 μg/mg) and by 83% (to 3.3 ± 1.7 μg/mg) after 48 h in the northern population. The southern population decreased sorbitol levels 64% (11.8 ± 0.69 to 4.2 ± 0.62) after 48 h. The southern population resynthesized more sorbitol than did the northern population upon re-exposure to 0°C. Glycerol levels increased linearly during the experimental period independent of temperature.

INTRODUCTION

The acquisition of cold hardiness is, with few exceptions (Morrissey and Baust, 1976; Somme, 1965), characterized by the seasonal accumulation of low molecular weight antifreeze/cryoprotective agents (polyhydric alcohols and saccharides) (see reviews by Salt, 1961; Asahina, 1966; Baust, 1973 and 1981; Somme, 1982). As these agents accumulate in species demonstrating high supercooling capacities, antifreezes function colligatively by depressing both haemolymph freezing and whole-body supercooling points (Lee *et al.*, 1981). Most species with high supercooling capacities are freeze susceptible. Conversely, most freeze-tolerant species accumulate high concentrations of polyhydroxy solutes but exhibit limited supercooling capacities. Recently, it has been observed that the actions of ice nucleating compounds/structures have a mitigating effect on supercooling that reduces and even eliminates colligative influences (Somme, 1978; Lee *et al.*, 1981; Baust and Zachariassen, 1983). In these species, polyhydroxy agents are presumed to serve cryoprotective functions (Baust, 1973).

The overwintering third-instar larvae of *Eurosta solidaginis* (Fitch) (Diptera, Tephritidae) is freezing tolerant and distributed between 27° and 54°N latitude on the North American continent. Northern as compared to southern populations experience distinctly different winter conditions resulting in separative adaptive strategies (Baust *et al.*, 1979; Baust and Lee, 1981 and 1982; Baust, 1983). All populations accumulate two principal cryoprotective agents (glycerol and sorbitol and three secondary agents (fructose, glucose and trehalose)) (Morrissey and Baust, 1976; Storey *et al.*, 1981; Baust and Lee, 1981 and 1982). Chilling to temperatures below 8°C serves as a trigger to sorbitol synthesis (Baust and Lee, 1982); however, glycerol accumulations occur independent of thermal conditions (Baust, 1983).

While qualitative similarities exist in the nature of polyhydroxy compounds found between populations representing latitudinal extremes, northern and southern groups reveal opposite patterns of supercooling. Northern populations reduce supercooling capacity to −6.2°C during cold hardening while southern populations increase supercooling capacity to −14.2°C. The differences in supercooling capacities are apparently due to the synthesis of haemolymph-borne ice-nucleating proteins in the northern population (Lee *et al.*, 1982).

The purpose of this study was 2-fold. First, the de-hardening strategies displayed by separate populations have been analyzed with respect to polyhydroxy catabolism and re-synthesis potential. The time course of de-hardening that partly characterizes warm acclimation and attendent reacclimation capabilities during periods of "spring" chilling are described. Secondly, the termal dependency and cycling ability of sorbitol and the simultaneous independence of glycerol metabolism have been quantified.

MATERIALS AND METHODS

Hardening–de-hardening experiment

Third-instar larvae from southern populations were collected during early October before exposure to environmental trigger temperatures. Specimens were placed at 20, 15, 10, 5, 0 or −5°C for four days and then exposed to 20°C for an additional seven days.

Both experimental procedures required approximately 4000 larvae. All specimens were maintained at 95–100% relative humidity in total darkness.

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De-hardening experiments

Winter-acclimated third-instar larvae of *Eurosta solidaginis* were collected during periods of peak cryoprotectant accumulation (northern populations—late December; southern populations—late January). Northern populations were collected in central Ohio and maintained at −25°C for four weeks. Southern populations were collected along the southeast Texas Coastal plain. Both populations were then maintained at −12°C for 3–4 weeks to allow for the acquisition of baseline characteristics before experimentation. The experimental protocol consisted of removing the larvae from the plant gall tissue and placing the specimens in covered petri plates containing water moistened filter paper. Following varied intervals at 20°C (0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3–10 days), a portion of each sample population was exposed to 0°C for seven days.

Cryoprotectant analysis

Whole body levels of low molecular weight sugars and sugar alcohols (<500 daltons) were measured using high performance liquid chromatography. Approximately 3 samples of 10 larvae each (~450 mg) were assayed per sampling interval. Details of extraction and analysis have been reported elsewhere (Baust and Edwards, 1979; Hendrix *et al.*, 1981). All data are expressed as μg/mg wet weight ± standard error of the mean.

RESULTS

Both northern and southern populations demonstrated rapid but reversible losses of sorbitol upon exposure to 20°C (Fig. 1). During the first 12 h, sorbitol levels decreased by 36% (19.7 ± 0.6 to 12.6 ± 1.2 μg/mg) and after 48 h by 83% (to 3.3 ± 1.7 μg/mg) in northern specimens. Southern populations had slightly lower initial levels of sorbitol (11.8 ± 0.69 μg/mg) which may have been due to difference in the low temperature acclimation protocol used between the two populations (Baust and Lee, 1982). After 2 days at 20°C, levels decreasd by 64% (to 4.2 ± 0.62 μg/mg) in the southern population. Following this interval, both populations maintained approximately equivalent sorbitol levels. During days 3 through to day 10 the basal levels of sorbitol for northern (1.4 μg/mg) and southern (1.6 μg/mg) populations were similar ($P > 0.5$). Figure 2 illustrates the qualitative similarity of sorbitol resynthesis (re-aclimation to cold) for both populations. Following each interval of exposure to 20°C longer than 24 h, chilling to 0°C for seven days resulted in sorbitol accumulatoin. Even daily intervals following day 3, the minimal period required for complete warm acclimation, and through day 8, both populations retained their abilities to resynthesize sorbitol to respective base levels upon re-exposure to 0°C. For the northern population the 0°C base was 6.3 μg/mg versus 10.5 μg/mg for the southern groups. Interestingly, re-acclimation resulted in the attainment of a 67% higher basal level in the southern population.

Glycerol levels were not substantatively affected by either warm acclimation or re-aclimation to cold (Figs 1 and 3). In the northern population glycerol levels diminished only 4% after 10 days at 20°C (30.4 ± 1.9 to 29.1 ± 0.2 μg/mg) ($P > 0.5$) and by 10% in the southern population (23.4 ± 1.2 to 21.0 ± 1.9 μg/mg) ($P > 0.2$). Mean 20°C basal levels of glycerol (days 3–10) were 27.3 and 21.7 μg/mg for northern and southern groups, respectively. Re-acclimation at 0°C for 7 days resulted in a slight elevation of basal levels to 29.1 ± 0.2 (northern) and 22.8 ± 0.36 μg/mg (southern) or a 6% increase ($P > 0.1$). The changes in glycerol levels during either de-hardening or re-acclimation to cold were not significant for either population.

Trehalose levels diminished abruptly following 6 h at 20°C in the northern population (Fig. 1) (18.0 ± 0.8 to 14.7 ± 0.5 μg/mg). However, throughout the remainder of the 10-day interval, trehalose levels were

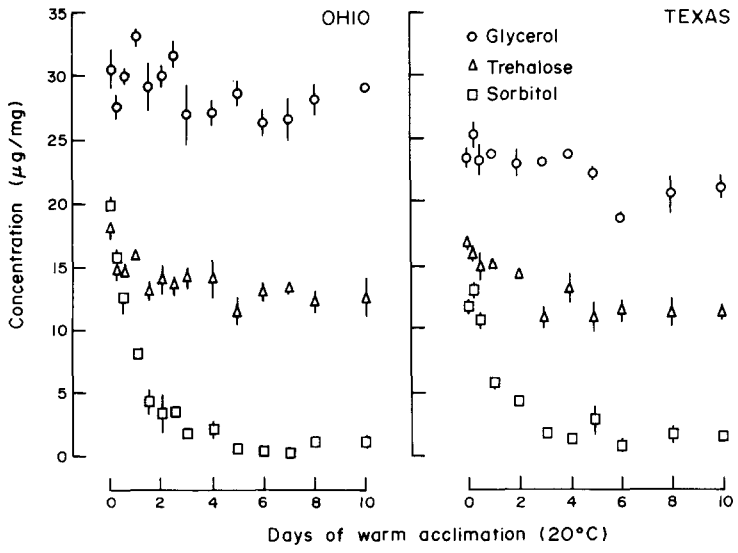

Fig. 1. Effects of warm acclimation (20°C) on antifreeze/cryoprotectant turn-over in cold hardened northern (Ohio) and southern (Texas) population of *Eurosta solidaginis*.

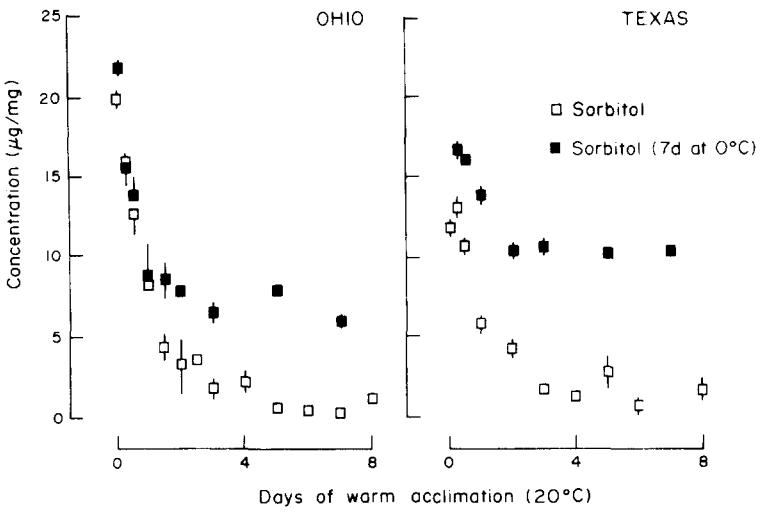

Fig. 2. Effects of warm acclimation (20°C) and re-acclimation to cold (7 days at 0°C) on sorbitol content in two populations of *Eurosta solidaginis*.

gradually reduced to basal levels of 12.9 (northern) and 11.5 μg/mg (southern). Re-acclimation to 0°C resulted only in an elevation of basal levels to 13.5 (northern) and 13.2 μg/mg (southern) which were not significant ($P > 0.5$).

The acute temperature sensitivity and dependency of sorbitol metabolism is illustrated in Fig. 4. Exposure of early autumn larvae (southern population) to temperatures below 10°C resulted in the initiation of sorbitol synthesis. Between 0.5 and 2 days of exposures to 5, 0 or −5°C, the rates of sorbitol production were equivalent. Between days 2 and 4, the 5 and −5°C rates of increase were identical. However, the 0°C rate of increase was over four times greater suggesting a sharply defined optimum. After 4 days, all specimens were returned to 20°C. Those initially exposed to 0°C immediately began to catabolize sorbitol whilst those exposed to 5°C continued to produce sorbitol for an additional 6 h. Those initially exposed to −5°C continued production for 24 h at 20°C.

Glycerol metobolism was not dependent on low temperature exposure (Fig. 5). At each temperature below 20°C, glycerol fluctuations were less than control value ($P > 0.025$) (single classification Anova).

DISCUSSION

Sorbitol synthesis in the southern population of *E. solidaginis* is initiated upon low-temperature exposure (Fig. 4). The triggering temperature for sorbitol induction is < +10°C, while the optimal rate of sorbitol synthesis occurs at 0°C. A relatively high temperature due to sorbitol induction among southern populations provides an anticipatory (adaptive) advantage that facilitates the hardening response prior to the occurrence of frost conditions. This population responds to

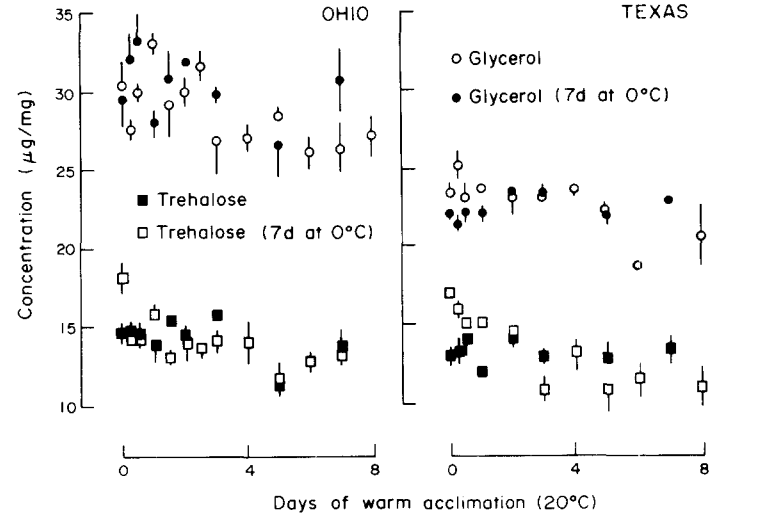

Fig. 3. Effects of warm acclimation (20°C) and re-acclimation (7 days at 0°C) on glycerol and trehalose content in two populations of *Eurosta solidaginis*.

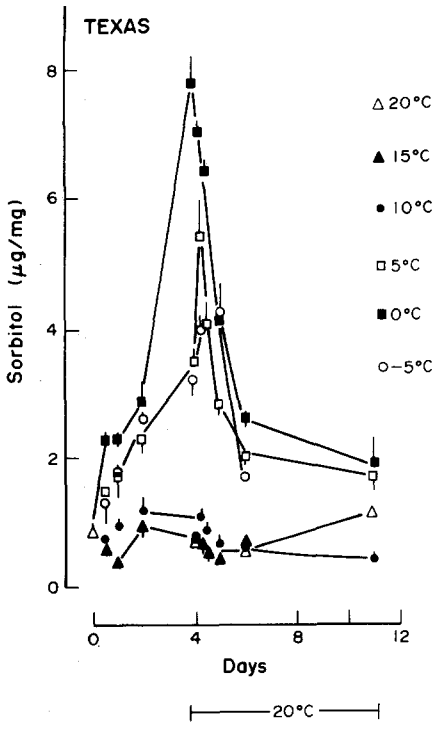

Fig. 4. Dependent effects of temperature cycling on sorbitol levels in a southern population of *Eurosta solidaginis*.

warm acclimation following cold exposure by rapidly catabolizing sorbitol to basal levels. Upon return to +20°C, sorbitol levels continue to rise for 6 to 24 h in larvae initially acclimated to +5 and −5°C before catabolism. This overshoot is best explained as a gradual turning off of the sorbitol cellular synthesizing machinery concomittant with a gradual turning on of the sorbitol degrading machinery. It is not uncommon in biological systems for some time to elapse before the cellular adjustments are made, and very often, a process will show an overshoot before reversing itself. Both northern and southern cold-hardy *E. solidaginis* respond to warm acclimation by rapidly catabolizing sorbitol to basal levels (within 48 h) (Fig. 2). In addition, both populations were able to resynthesize the sorbitol lost during exposure to +20°C upon return to 0°C. Temperature modulation of sorbitol synthesis in *E. solidaginis* has been investigated on an enzymatic level (Storey and Storey 1981; Storey 1982). Their data suggest that in a northern population of *E. solidaginis*, three key enzymes of carbohydrate catabolism increase in activity in response to low temperature acclimation: phosphorylase, hexokinase and phosphofructokinase. In addition, the activities of polyol dehydrogenase and sorbitol dehydrogenase increased as acclimation temperature decreased. These activity changes closely parallel the low temperature accumulation of sorbitol. Polyol dehydrogenase catalyzes the conversion of glucose to sorbitol and sorbitol dehydrogenase, the conversion of fructose to sorbitol. Storey (1982) found that in this species phosphofructokinase is strongly inhibited by low temperature and that by its inhibition, carbon flow is diverted from glycerol production to that of sorbitol. The southern population of *E. solidaginis* initiated sorbitol synthesis following one day at 5°C, while northern populations required a period in excess of one but less than five days to attain equivalent levels (Baust, 1982). There is a difference in the triggering kinetics of sorbitol synthesis between the two populations, and from this study, a difference in the response time of that machinery of reacclimation. It is expected that the environmental cues to cold-hardening and diapause termination would be different between northern and southern populations in order to guarantee survival.

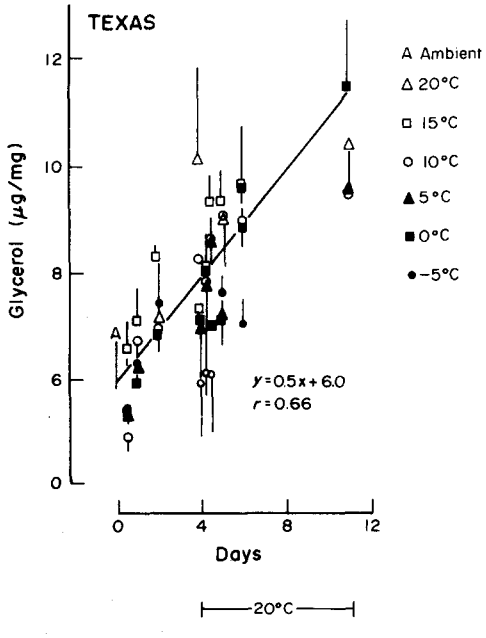

Fig. 5. Independence of temperature cycling on glycerol levels in a southern population of *Eurosta solidaginis*.

Glycerol metabolism in southern *E. solidaginis* is independent of temperature. Glycerol levels increased over the 11-day period regardless of temperature and continued to rise on transfer to 20°C (day 4) (Fig. 5) ($y = 0.5X + 0.60$) ($r = 0.66$). Baust and Morrissey (1976) reported that following cold-acclimation in a New York population of *E. solidaginis* glycerol levels were maintained at nearly constant levels independent of temperature. Baust and Miller (1970) showed that in a carabid beetle, *Pterostichus brevicornis*, glycerol synthesis is temperature dependent. Winter beetles collected at an ambient temperature of −10°C and rapidly warmed to +20°C lost all glycerol within 36 hr. This loss was irreversible i.e. return to low temperatures did not induce re-synthesis. One explanation for the linear increase in glycerol independent of temperature is that in this species glycerol is induced by a stress other than temperature. Once initiated, glycerol synthesis continues independent of other environmental perturbations. Perhaps removal of the larvae from its gall is a sufficient "stress" to initiate glycerol synthesis. Water stress has been preliminarily implicated in glycerol production in this species. Studies by Baust (1983) demonstrate a correlation between desiccation and glycerol build-up; glycerol levels were calculated on an initial wet weight basis to negate

concentration effects due to water loss. Enzymes involved in carbon flow from glycolysis to the tricarboxylic acid cycle may be "water sensitive". When a "critical" water ratio (free to bound) is reached these enzymes may shutdown thereby diverting carbon flow to glycerol production. Although the percentage of water in *E. solidaginis* remains constant (~65–70%) larval weight falls during late autumn and winter resulting in a reduction of the absolute amounts of water in each larvae. Also, Storey *et al.* (1981) have shown that the amount of "bound-water" as opposed to bulk-water increases during winter and causes an effective loss in water available for metabolic functions. Bound water was shown to increase directly with decreased acclimation temperature. The increases were attributed to accumulation of small molecular weight (polyols, amino acids, etc.) compounds and also to qualitative changes in high molecular weight (proteins, glycogen) components. Young and Block (1980) reported that in the Antarctic mite *Alaskozetes antarcticus* both low temperature acclimation and desiccation increased glycerol concentrations.

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