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Seasonal changes in fatty acid composition associated with cold-hardening in third instar larvae of *Eurosta solidaginis*

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Abstract Third-instar larvae of the goldenrod gall fly *Eurosta solidaginis* (Diptera: Tephritidae) survive extended periods in winter during which tissue water is frozen. Both low temperature and reduced water activity during freezing present challenges for the structural integrity of cellular lipids. Fatty acids of both phospholipids and triacylglycerols from fat body cells of *E. solidaginis* were analyzed throughout fall and early winter, a period that encompasses the acquisition of freeze-tolerance, to determine if adaptations to freezing include changes in fatty acid unsaturation. The five most abundant fatty acids from both fractions were (in decreasing order) oleic (40–65%), palmitoleic (18–20%), palmitic (12–17%), linoleic (5–10%), and stearic acids (4–7%). This represents a typical complement of Dipteran fatty acids, although oleic acid levels were higher in *E. solidaginis* than those reported from other Dipterans (~28%; Downer 1985). From September to November, monounsaturates increased from 59 to 70% in phospholipids at the expense of saturated fatty acids (25%–20%) suggesting activation of a Δ^9-desaturase enzyme. These changes resulted in an increase in the ratio of unsaturated to saturated fatty acids (U/S) from 3.0 to 4.2, although there was no change in the average number of double bonds per fatty acid (unsaturation index, UI ≈ 1.2 in phospholipids and 0.9 in triacylglycerols throughout the season). These changes were temporally correlated to decreasing ambient temperatures and increasing larval and fat body cell freeze-tolerance.

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Introduction

Adaptive changes in the composition of cellular lipids are a widespread feature of thermal adaptation in ectothermic animals (review: Hazel 1988, 1989). Compositional changes that preserve the fluidity of lipids and lower the liquid crystal-to-gel phase transition temperature are broadly termed homeoviscous adaptation (Sinensky 1974), and are well documented in non-freezing ectotherms. Few studies, however, have addressed the adaptations in lipids that accompany the onset of freeze-tolerance in animals that freeze as a normal part of their life histories. This is particularly notable as cell membranes are the major site of freeze damage in freeze-susceptible species and in tissues preserved by freezing (Steponkus and Lynch 1989). Frozen tissues must compensate for both the effects of temperature on membrane order and the effects of dehydration resulting from decreased water activity. Freeze-induced dehydration lowers the lamellar-to-hexagonal phase transition temperature of natural bilayers (Webb et al. 1994), resulting in deterioration of the membrane's barrier properties. Also, cell shrinkage due to osmotic water loss following dehydration is accompanied by loss of membrane material via endocytic vesiculation in some freeze-susceptible species, and may be irreversible (Webb et al. 1994).

The few studies that have addressed adaptations to freezing of animal cell lipids report changes in fatty acid composition with the onset of freeze-tolerance. For example, cold hardening in the barnacle *Balanus balinoides* is accompanied by an increase in fatty acid desaturation in both phospholipids and neutral lipids (Tooke and Holland 1985; Tooke et al. 1985). The literature on membrane adaptations in plants is more extensive. Freeze-tolerance in potato leaves is associated with an

increase in dienoic fatty acids at the expense of saturates in membrane phospholipids, among other lipid adaptations (Palta et al. 1993). Introduction of the gene for the unsaturate-specific lipid-synthesizing enzyme glycerol-3-phosphate acyltransferase from chill-resistant *Arabidopsis* confers chilling resistance to the normally chilling-sensitive tobacco plant (Murata et al. 1992). In those species that have been studied, an increase in fatty acid unsaturation appears to be an important feature of freeze-tolerance, as is well documented in cold-acclimated, freeze-susceptible animals.

The goldenrod gall fly *Eurosta solidaginis* (Diptera: Tephritidae), which overwinters as a freeze-tolerant third instar larvae encased in stem galls of goldenrod, has been extensively used as a metazoan model for freeze-tolerance (reviews: Lee 1991; Lee et al. 1995; Storey and Storey 1988). The onset of freeze-tolerance occurs sometime in the fall, depending upon the geographic location of the population (Baust et al. 1979; Bennett 1995). Joanisse and Storey (1996) report a significant increase in the monoene, 18:1(n-9) oleic acid, at the expense of the saturates, palmitic acid (16:0) and stearic acid (18:0), in fully freeze-tolerant larvae collected in December and March versus freeze-susceptible larvae collected in September. They do not, however, distinguish between the fatty acid composition of membrane phospholipids and the more abundant storage lipid fraction, predominantly triacylglycerols. In this study, we examined seasonal changes in fatty acid composition of both the phospholipid and triacylglycerol fractions associated with the development of freeze-tolerance in fat body cells from *E. solidaginis*. Fat body cells are of particular interest because they not only synthesize cryoprotectants, but have been demonstrated to survive intracellular freezing and a dramatic rearrangement of their cytoplasmic contents upon thawing (Lee et al. 1993; Morason et al. 1994). Simultaneous studies on the survival of both whole larvae and isolated fat body cells indicate that the onset of freeze-tolerance occurs in late September and early October in this population, and that by October 10 all whole larvae and fat body cells are able to withstand freezing at −10 °C (Bennett 1995). Changes in the levels of unsaturated fatty acids in both phospholipids and triacylglycerols may contribute to low temperature adaptation of membranes and fuel reserves, respectively, in this freeze-tolerant animal.

Materials and methods

Spherical galls from goldenrod (*Solidago* sp.) containing third instar larvae of *Eurosta solidaginis* were collected from fields at Miami University's Ecology Research Center once every 2 weeks from mid-August through mid-December and once at the end of January 1994–95. During this sampling period, ambient field temperatures were monitored by the Miami University weather station at the Ecology Research Center. During August and September mean daily ambient temperatures were between 15 and 20 °C, while October values decreased to approximately 15 °C (Bennett 1995). In November, mean values were between 5 and 10 °C. Weekly means decreased to between −3 and 5 °C in December and January.

For each sample, 8–10 mg of fat body cell (FBC) tissue was dissected and pooled from six larvae. Ten larvae per sample were used in August due to low larval weight. Five samples were prepared from each collection. Lipids were extracted using the technique of Bligh and Dyer (1959) as modified by Garbus et al. (1963) and stored as a biphasic system under N_2 at −20 °C for 2–7 months until analyzed.

Polar and neutral complex lipids were separated using thin layer chromatography according to Christie (1982) on silica gel H (Sigma, St. Louis, Mo.; 0.5 mm thickness) in a solvent system of hexane/diethyl ether/formic acid (80:20:2). Lipids were visualized in iodine vapor, and spots corresponding to phospholipids and triacylglycerols were scraped into 2 ml 2.5% H_2SO_4 in dry methanol. Fatty acids were transesterified to methanol via acid-catalyzed metholysis (70 °C for 2 h), and fatty acid methyl esters (FAME) were extracted into hexane using three washes of 2 ml each. The hexane washes were pooled for each sample and dried under nitrogen gas. FAME were redisolved in 10 µl of hexane. One microliter of this solution was injected into a Hewlett-Packard 5890 gas-liquid chromatograph (GLC) fitted with a flame ionization detector and a 32-foot SP-2330 wall-coated open tubular capillary column (Supelco, Bellefonte, Pa., USA). Fatty acids were identified on the basis of comparisons of corrected relative retention times to those of authentic standards (Sigma). Seasonal changes in arcsin transformed lipid percentage data were analyzed over time using one way analysis of variance (ANOVA), and Tukey-Kramer multiple comparisons post-test when the resulting F statistic was considered to be significant ($P \leq 0.05$).

Results

The dates corresponding to the onset of freeze-tolerance were established by Bennett (1995) in animals of the same population collected on the same dates. Of 20 animals collected on September 26, none responded (showed signs of peristaltic muscular contraction) to stimulus following 24 h at −25 °C. One hundred percent of the 20 animals collected on October 10 were responsive following the same freezing regime. The 2 weeks between these dates were thus identified as a critical period for the onset of cold-hardening in this population.

Phospholipids

Seven major fatty acids dominated the phospholipids of fat body cells from *Eurosta solidaginis*. The unsaturated fatty acids, oleic acid [18:1(n-9)], palmitoleic acid [16:1(n-7)], linoleic acid [18:2(n-6)], and linolenic acid [18:3(n-3)] accounted for over 60% of the total fatty acids in phospholipids and over 70% in triacylglycerols (Fig. 1A). Three saturated fatty acids, palmitic acid (16:0), stearic acid (18:0), and myristic acid (14:0), represented about 25% of the phospholipid fatty acid, and 20% of the triacylglycerol fatty acid throughout the duration of the study (Fig. 2A). Other fatty acids found in trace amounts (<1%) included 11:0, 12:0, 13:0, 15:0, 17:0, 20:0, 14:1, 14:2, and 18:1(n-13).

Seasonal changes in individual fatty acids are discussed in order of decreasing relative abundance. Oleic acid [18:1(n-9)], which was the most abundant fatty acid,

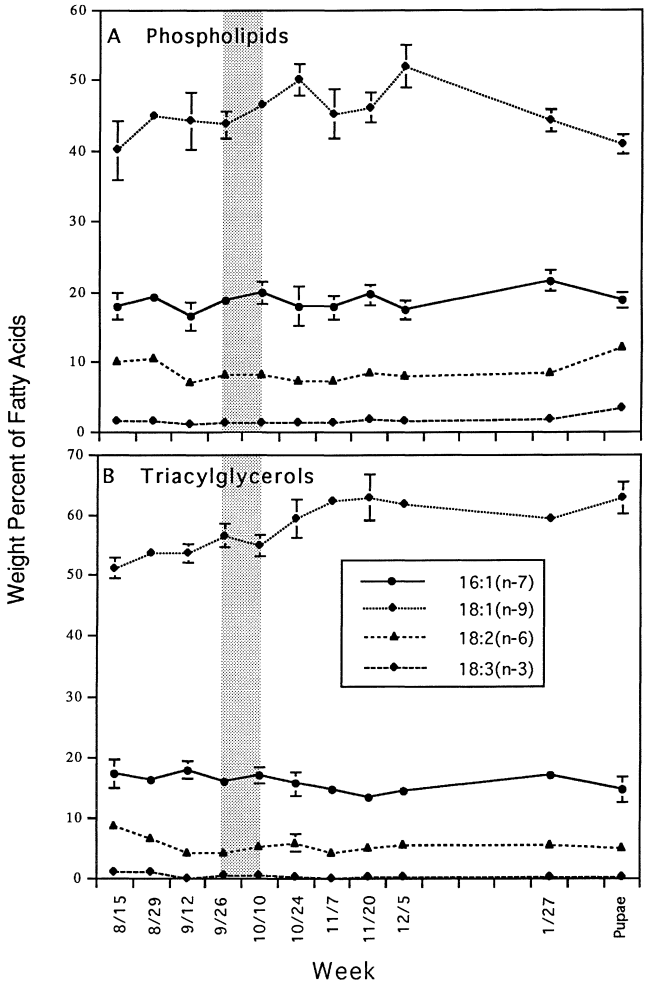

Fig. 1A, B Seasonal changes in weight percent (n = 5) of unsaturated fatty acids: palmitoleic acid [16:1(n-7)], oleic acid [18:1(n-9)], linoleic acid [18:2(n-6)] and linolenic acid [18:3(n-3)] from (**A**) phospholipid and (**B**) triacylglycerol fractions of fat body cells from *Eurosta solidaginis* third instar larvae. *Shaded bar* represents the weeks during which larvae become freeze-tolerant

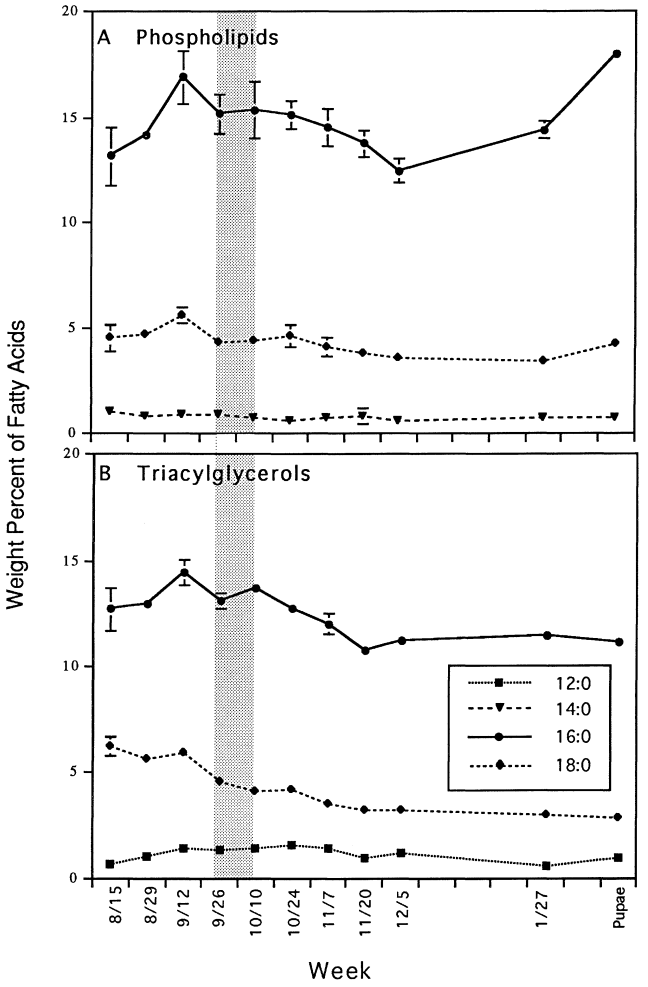

Fig. 2A, B Seasonal changes in weight percent (n = 5) of saturated fatty acids: lauric acid (14:0), myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0) from (**A**) phospholipid and (**B**) triacylglycerol fractions of fat body cells from *Eurosta solidaginis* third instar larvae. *Shaded bar* represents the weeks during which larvae become freeze-tolerant

was lowest in August (40.1 ± 4.2%) and highest in December (52.0 ± 3.1%), a 30% increase. The levels of oleic acid rose between September 26 and October 10, the critical period for cold-hardening. The proportion of oleic acid fell back to 40.8 ± 1.4% again in the pupal stage (Fig. 1A). The increase in oleic acid levels as a function of collection date during the autumn cold-hardening season (August–December) were significant (r = 0.38, slope significantly different from zero at P = 0.0078).

The second most abundant fatty acid was palmitoleic acid [16:1(n-7)], which ranged from 16.5 to 21.5%, but did not change significantly during the season (Fig. 1A, Table 1). Palmitic acid (16:0), on the other hand, increased from 13.1 ± 1.4% in mid-August, peaked at 16.9 ± 1.2% on September 12 and then decreased through the autumn to 12.4 ± 0.5% by early December. Palmitic acid was much higher again (18.0 ± 0.3%) in the pupal stage (Fig. 2A, Table 1).

The weight percent of linoleic acid [18:2(n-6)] was higher in August and in the pupal stage (10–12%) than it was during the rest of the season when it remained constant around 7–8% (Fig. 1A). Stearic acid (18:0) decreased significantly from September (5.6 ± 0.4%) to January (3.5 ± 0.2%; Table 1). Pupae contained intermediate proportions (4.3 ± 0.2%) of this fatty acid (Fig. 2). Linolenic acid [18:3(n-3)] comprised 1.2–1.8% of phospholipid fatty acids in larvae throughout the season, but increased three-fold in the pupal stage (3.5 ± 0.2%; Fig. 1A, Table 1). Finally, myristic acid (14:0), although found in relatively low abundance, decreased from September (1.2 ± 0.3%) to December (0.5 ± 0.1%; Fig. 2A, Table 1).

These results can be summarized by considering three major structural classes of fatty acids: saturates, monoenes, and polyunsaturates. Overall, monoenoic fatty acids accounted for the majority of fatty acids from phospholipids. They were lowest in August (58.7 ± 5.4%)

Table 1 Results of one-way analysis of variance (ANOVA) for seasonal changes in arcsin transformed lipid data from phospholipid and triacylglycorol fractions of *Eurosta solidaginis* fat body cells

	Phospholipids		Triglycerides	
	F	*P*	*F*	*P*
Fatty acids:				
16:1 (n-7)	0.75	0.68	0.95	0.5
18:1 (n-9)	1.71	0.10	5.42	< 0.01
18:2 (n-6)	4.36	< 0.01	2.14	0.04
18:3 (n-3)	11.64	< 0.01	–	–
12:0	–	–	1.19	0.33
14:0	2.60	0.01	–	–
16:0	3.09	< 0.01	6.11	< 0.01
18:0	2.79	< 0.01	32.68	< 0.01
Classes of fatty acids:				
Saturates	1.20	0.31	23.56	< 0.01
Monoenes	1.39	0.21	14.92	< 0.01
Polyunsaturates	5.72	< 0.01	2.36	0.03
Indices of unsaturation:				
U/S	1.17	0.34	30.51	< 0.01
UI	0.97	0.48	4.01	< 0.01

and highest in December (69.4 ± 2.3%; Fig. 3A), a pattern attributed primarily to the apparent increase in the proportion of oleic acid [18:1(n-9); Fig. 1A]. During this same time interval, the proportion of all saturated fatty acids was highest in September (27.6 ± 3.7%) and lowest in December (19.5 ± 1.6%), reflecting the decrease in both palmitic acid (16:0) and stearic acid (18:0; Fig. 2A). Although ANOVA was not sensitive enough to detect significant seasonal changes in these two classes of fatty acids (Table 1), regression did detect a significant increase in monoenes ($r = 0.28$, $P = 0.0408$) and a simultaneous decrease in saturates ($r = -0.27$, $P = 0.0496$) during the season. The weeks in late September and early October, at the onset of freeze-tolerance, marked an increase in monoenes and decline in saturates and not a plateau in either class of fatty acid. Polyunsaturates were the least abundant class of fatty acids, and reflected the changes seen in linolenic acid [18:3(n-3); Fig. 1A], i.e., significantly higher in August larvae and the pupal stage (12–15%) than in larvae during the rest of the season, when polyunsaturates accounted for a constant 8–10% of all fatty acids (Fig. 3A, Table 1).

Two indices were calculated to summarize overall changes in saturation. The ratio of unsaturated to saturated fatty acids (U/S), which was the cumulative weight percent of all unsaturated fatty acids divided by the cumulative weight percent of all saturated fatty acids, was lowest in September (2.8 ± 0.5) and highest in December (4.2 ± 0.4; Fig. 4A), reflecting the simultaneous increase in monoenes and decrease in unsaturated fatty acids (Fig. 3A). The unsaturation index (UI), which is the average number of double bonds per fatty acid [(weight percent of each fatty acid × number of double bonds)/100], remained constant at 0.8–0.9 throughout the season (Fig. 4A).

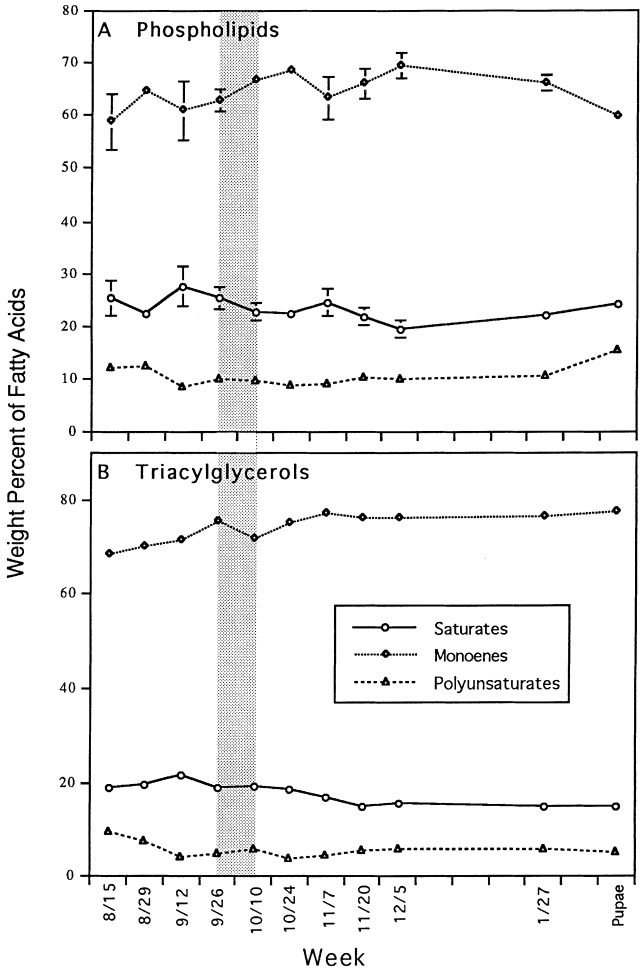

Fig. 3A, B Seasonal changes in weight percent ($n = 5$) of classes of fatty acids from (**A**) phospholipid and (**B**) triacylglycerol fractions of fat body cells from *Eurosta solidaginis* third instar larvae. *Shaded bar* represents the weeks during which larvae become freeze-tolerant

Triacylglycerols

Six fatty acids dominated the triacylglycerol fraction of fat body cell lipids, five of which were the same and in the same order of abundance as those found in the phospholipid fraction. These comprised the unsaturated fatty acids, oleic acid [18:1(n-9)], palmitoleic acid [16:1(n-7)] and linoleic acid [18:2(n-6); Fig. 1B], and the three saturated fatty acids, palmitic acid (16:0), stearic acid (18:0) and lauric acid (12:0; Fig. 2B). Linolenic acid [18:3(n-3)] and myristic acid (14:0) were also present in triacylglycerols, but in trace amounts (>1%).

Oleic acid [18:1(n-9)], which again was the most abundant fatty acid, increased significantly from 51.2 ± 1.7% in August to 63.0 ± 0.8% by late November (Table 1). Simultaneously, stearic acid (18:0) decreased by almost 50% from 6.2 ± 0.4% to 3.2 ± 0.1% where it remained constant for the rest of the winter (Fig. 2B, Table 1). Palmitic acid (16:0), after peaking at 14.4 ± 1.3% in early September, decreased significantly to its winter level of 10.8 ± 0.5% by late November

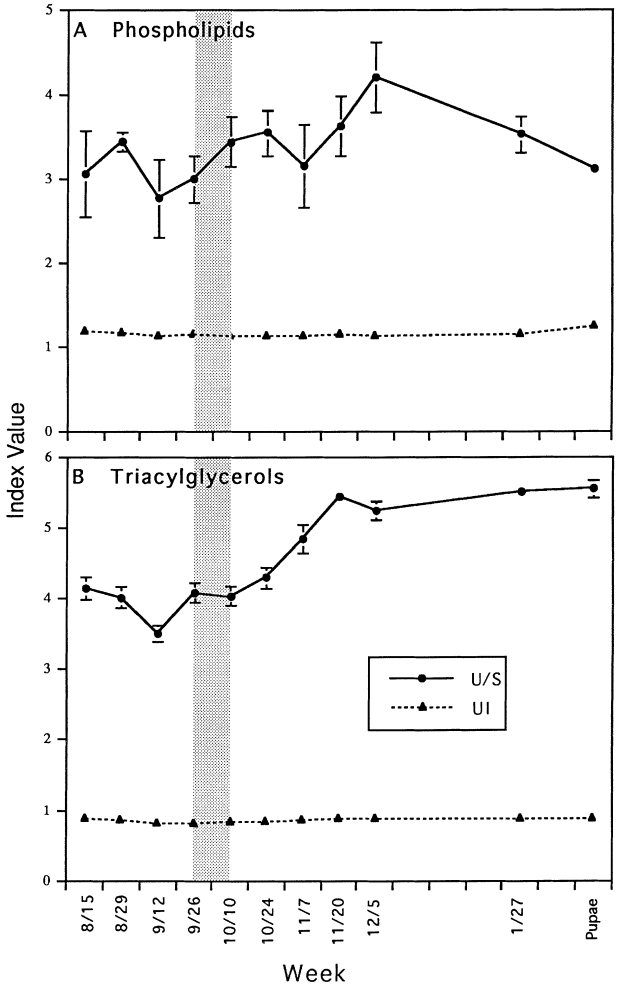

Fig. 4A, B Seasonal changes in the unsaturated:saturated ratio [(weight percent of monoenes + polyunsaturates)/weight percent of saturates] of fatty acids ($n = 5$) and the unsaturation index (average number of double bonds per fatty acid; UI) of fatty acids ($n = 5$) from (**A**) phospholipid and (**B**) triacylglycerol fractions of fat body cells from *Eurosta solidaginis* third instar larvae. *Shaded bar* represents the weeks during which larvae become freeze-tolerant

(Fig. 2B, Table 1). All other fatty acids remained constant during the course of the study (Figs. 1B, 2B, Table 1). The proportions of the various fatty acids in the pupal stage were similar to those found in the larval stage in late January (Figs. 1B, 2B).

These changes resulted in an overall increase in the proportion of monounsaturated fatty acids during the course of the season from 68.3 ± 0.7% to 77.1 ± 0.8% and an overall decrease in the proportion of saturated fatty acids from 21.7 ± 0.6% in September to 15.0 ± 0.2% in January (Fig. 3B, Table 1). Polyunsaturates decreased slightly in August but remained steady around 4–6% for the rest of the season (Fig. 3B). The ratio of unsaturated to saturated fatty acids (U/S) from triacylglycerols, although much higher than that for phospholipids, also rose sharply from September (3.5 ± 0.1) through November (5.4 ± 0.1) where it remained for the winter (Fig. 4B, Table 1). However, the average number of double bonds per fatty acid (UI) did not change (0.8–0.9). Their level was the same for both triacylglycerols and phospholipids (Fig. 4).

Discussion

Destabilization of cellular membranes is the primary cause of freeze-thaw damage in organisms that either do not freeze as a normal part of their life cycles, or those that do but are not sufficiently acclimatized to viably enter the frozen state (review: Steponkus and Lynch 1989). Changes in membrane lipids that accompany the acquisition of freeze-tolerance have been studied to determine if there is a unique composition of the freeze-tolerant cell membrane. One recurring theme in studies of membrane composition is apparent: the change from the freeze-susceptible to the freeze-tolerant state involves changes in the proportions of existing lipid species and not the appearance of unique lipids. The fatty acids of *Eurosta solidaginis*, both before and after the onset of freeze-tolerance in the fall, are typical of those reported for other Dipterans (Downer 1985), although the proportions differ. In the phospholipids and triacylglycerols reported in the present study, and in Downer's report of total lipids of Dipterans in general, fatty acids occur with the relative abundance of 18:1 > 16:1 > 16:0 > 18:2 > 18:0 > 18:3 ≈ 14:0. [Linolenic acid (18:3), while detectable in the phospholipid fraction, was seen only in trace amounts in the triacylglycerols of *E. solidaginis*]. Throughout the fall, and encompassing the period of change in physiological state that marks the onset of freeze-tolerance in late September and early October (Bennett 1995), the U/S ratio steadily increased. This is attributed to the steady rise (up to mid-December) of the monoene 18:1(n-9) at the expense of the saturates 16:0 and 18:0. Joanisse and Storey (1996) reported a similar increase in 18:1(n-9) in the total lipid fraction in December and March relative to September levels in *E. solidaginis* collected from populations around Ottawa, Canada, also with a concurrent decline in the same saturates, 16:0 and 18:0. The overall fatty acid profiles from the Canadian population were very similar to those reported here, although the presence of the short-chain saturates, 12:0 and 14:0, found at low levels in the triacylglycerol and phospholipid fatty acids, respectively, in the Ohio population, were not reported in the Canadian population. Tooke and Holland (1985) also found that the fatty acid profiles of freeze-tolerant and freeze-susceptible barnacle genera were typical of related crustaceans, and that both freeze-tolerant and freeze-susceptible barnacles responded to the onset of winter by increasing (although only slightly) monoenes. These authors went on to study the fatty acid compositions of different subcellular membranes and of individual phospholipid classes, and found more pronounced increases in overall unsaturation of mitochondrial membranes and of the plasma membrane-specific lipid, sphingomyelin, in winter (Tooke et al. 1985).

There is evidence that these seemingly minor changes in phospholipid fatty acids can, in fact, confer major changes in the cryostability of cellular membranes, particularly if they represent retailoring of phospholipid molecular species; i.e., the arrangements of fatty acid pairs on individual phospholipids. At low water activities, the temperature at which phospholipid bilayers undergo lyotropic phase transitions from the lamellar to the hexagonal (H_{II}) state decreases to within the natural temperature range of the winter-acclimatized organism. H_{II} lipid structure is inconsistent with the barrier properties of the membrane and is accompanied by a concommitant leakage of electrolytes, loss of osmotic responsiveness upon thawing, and cell death (review: Hazel and Williams 1990; Steponkus and Webb 1992). Increased proportions of molecular species in which both fatty acids are unsaturated counteracts membrane destabilization via freeze-induced dehydration. Webb et al. (1994) report that protoplasts of freeze-tolerant rye have depressed lamellar-to-hexagonal phase tranisition temperatures relative to protoplasts prepared from freeze-susceptible oat. In both species, cold acclimation was accompanied by changes in membrane lipid composition that included increased proportions of phospholipid molecular species with two unsaturated fatty acids; the increase was greater in the freeze-tolerant rye than in freeze-susceptible oat. The subtle, time-dependent increase in fatty acid unsaturation observed in *E. solidaginis* could reflect more pronounced increases in the proportions of di-unsaturated phospholipid species, although further studies are required to establish this with certainty.

Another consequence of freeze-induced dehydration is cell shrinkage. As extracellular ice forms, the concentration of gases and solutes in unfrozen water increases resulting in osmotic water loss from cells. During osmotic shrinking, freeze-susceptible cells of rye leaves delete membrane material via endocytic vesiculation (Dowgert and Steponkus 1984). Upon thawing, expansion-induced lysis of the lipid-depleted membrane results in cell death. Changes in lipid composition that accompany cold-acclimation in freeze-tolerant organisms have been shown to prevent osmotically-induced endocytic vesiculation. Steponkus et al. (1988) have shown that fusion of protoplasts derived from freeze-susceptible rye leaves with liposomes either artificially prepared from mono- or diunsaturated phosphatidylcholine molecular species, or prepared from phospholipids of freeze-tolerant leaves, confers increased freeze-tolerance. Neither fused protoplasts nor cold-acclimated protoplasts lose membrane material by endocytosis, but instead form exocytotic extrusions that preserve membrane surface area and prevent expansion-induced lysis. The exact mechanism of compositional changes on the behavior of membranes, and whether these observations hold true for other freeze-tolerant species, is unclear.

The pattern of changes in the fatty acid composition of *E. solidaginis* triacylglycerols is very similar to that of the phospholipids in the fall and early winter (Figs. 1–3). Consequently, the overall unsaturation of depot lipids, as measured by U/S ratio, increases significantly throughout the fall, with the steepest increase leading up to and during the acquisition of freeze-tolerance in late September and early October, just as occurs in phospholipids (Fig. 4). Increased unsaturation of neutral lipids under cold conditions has been reported in several species of both freeze-tolerant and freeze-susceptible ectotherms (Hazel 1979; Tooke and Holland 1985; Ohtsu et al. 1993) and invariably has been interpreted by these authors as an adaptation that maintains the fluidity of storage fats to enhance their availability to energy-yielding enzymes. There is, however, little evidence that frozen *E. solidaginis* tap their abundant triacylglycerol reserves for energy. In fact, Joanisse and Storey (1996) report that the levels of triacylglycerols in this species remain constant throughout the winter and that the activity of the enzymes of fatty acid beta-oxidation falls dramatically. The fatty acid composition of triacylglycerols may reflect the composition of the intracellular fatty acid pool; i.e., the acids that are synthesized, desaturated and/or elongated under prevailing ambient conditions. As winter approaches, the intracellular pool of fatty acids may be desaturated for insertion into structural phospholipids for the important purpose of preventing freeze-injury and maintaining membrane fluidity. Thus, even randomly assembled neutral lipids would reflect increases in the unsaturation of the fatty acid pool from which they were constructed. This is consistent with the similar fatty acid profiles observed in phospholipids and triacylglycerols during the acquisition of freeze-tolerance.

The steady increase in 18:1(n-9) and concomitant decline in levels of 18:0 in *E. solidaginis* lipids imply an increase in the activity of a Δ^9 desaturase enzyme with decreasing temperature. While the activity levels of most enzymes decline with a decrease in temperature, the membrane-bound desaturases are activated under these conditions. Carp liver (Wodtke and Cossins 1995), *Neurospora* (Martin et al. 1981), rainbow trout (Hagar and Hazel 1985), *Brassica napus* (Williams et al. 1992), *Tetrahymena* (Umeki and Nozawa 1984) among others, exhibit increases in desaturase activity at low temperatures, although the mechanism, activation of existing enzyme by the direct effects of temperature on the enzymes' lipid micromilieu or gene induction, is not firmly established, and may differ in different organisms.

Changes in fatty acid composition are among several means of altering lipids in response to low temperature. Different phospholipid head groups are known to pack differently in a bilayer and influence the phase characteristics of membranes (Hazel and Williams 1990; Steponkus and Lynch 1989). Preliminary studies have shown that *E. solidaginis* in the freeze-tolerant state are unique in their low levels of choline-containing lipids (N.L. Pruitt, unpublished data), which are typically quite common in insects (Downer 1985). Therefore, a more detailed classification of phospholipid head groups from *E. solidaginis* is necessary to better understand the

mechanisms that prevent damage to cellular membranes in freeze-tolerant insects.

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