Internal lipids of sugarbeet root maggot (Tetanops myopaeformis) larvae: Effects of multi-year cold storage

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Sugarbeet root maggots, Tetanops myopaeformis (Diptera, Ulidiidae), survive more than five years of laboratory cold (6°C) storage as mature third-instar larvae. To quantify energy costs associated with prolonged storage, internal lipids of larvae stored for 1, 2, 3, and 5 years were compared and characterized with those of field-collected diapausing larvae. Internal lipid concentration was highest (21.8% wet wt. and 29.8% dry wt.) in diapausing larvae. Lipids decreased progressively over storage time with greater than 70% reductions for 5-year stored larvae. Thin-layer chromatographic analysis revealed that triacylglycerols (TAGs) were the most predominant class of internal lipids, with trace amounts of diacylglycerols and hydrocarbons also being present. Gas chromatography–mass spectrometry (GC–MS) analyses of TAG fractions identified ten major fatty acids (FAs). The proportion of unsaturated FAs was higher (73 to 78%) than saturated FAs in diapausing and stored larval groups. Palmitoleic acid (16:1) was the predominant FA, constituting 40–50% of total unsaturated FAs with lesser amounts of myristoleic (14:1), oleic (18:1), lauroleic (12:1), gadoleic (20:1), and the saturated FAs, palmitic (16:0), myristic (14:0), lauric (12:0), stearic (18:0), and arachidic (20:0) being detected at much lower concentrations. Characterization of intact TAGs by high performance liquid chromatography and GC–MS revealed the presence of more than 40 TAG constituents. In conclusion, TAGs are utilized as an important energy source for T. myopaeformis larvae during diapause and long-term cold storage with no observed impact of multi-year storage on the TAG composition and distribution of their fatty acids.

1. Introduction

Lipids and fat bodies have been the focus of many energy and metabolism-related studies in the areas of insect biochemistry and physiology. Lipids serve as a major source of energy reserves in many organisms, including insects. The overall evolutionary and ecological success of the class Insecta could be partly attributed to the group’s capacity for storing and using lipids as energy sources during periods of intense caloric demand such as migratory flight or prolonged non-feeding periods like diapause (Downer and Mathews, 1976). Diapause is a frequently encountered phenomenon in most insects inhabiting temperate climates. In addition to glycogen, lipids play an important role in energy supply and storage during diapause. The advent of modern chemical techniques has recently allowed scientists to identify lipid classes and characterize fatty acid composition, as well as determine the function of lipids in in vivo physiological and biochemical pathways.

Quantitative studies on lipids in insects have mainly been carried out to estimate energy costs associated with various physiological events such as embryogenesis, morphogenesis, reproduction, and diapause (Downer and Mathews, 1976). However, qualitative lipid studies in insects have been aimed more at the following: understanding various biosynthetic pathways (Arrese and Wells, 1994; Nor Aliza et al., 1999); investigating trophic relationships between insects and bacteria (Kiyashko et al., 2004); and determining the role of lipids in maintaining thermal homeostasis in low-temperature adapted insects (Ohtsu et al., 1993; Bennett et al., 1997). Triacylglycerols are the predominant class of lipids in insects, and they are the chief source of metabolic fuel in many overwintering insects (Adokon and Denlinger, 1985; Ohtsu et al., 1993). Fatty acid composition is not

Abbreviations: SBRM, sugarbeet root maggot; TAG, triacylglycerol; TIL, total internal lipids; TMS, trimethylsilyl; FAME, fatty acid methyl ester; BSA, N,N-bis-(trimethylsilyl) acetamide; DMDS, dimethyl disulfide; La, lauric acid; M, myristic acid; Mo, myristoleic acid; P, palmitic acid; Po, palmitoleic acid; S, stearic acid; O, oleic acid.

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fixed in insects, and it can change seasonally to serve the organism’s physiological needs during critical times for survival (Khani et al., 2007). Insects inhabiting low-temperature environments tend to have a higher proportion of unsaturated fatty acids to saturated fatty acids, which increases the fluidity of lipids, and has been shown to impart freeze tolerance in insects (Bennett et al., 1997). The association of increased unsaturated fatty acid concentrations and diapause initiation has been observed in Drosophila melanogaster (Ohtsu et al., 1993), Eurosta solidaginis (Fitch) (Bennett et al., 1997), Megachile rotundata (F.) (Buckner et al., 2004), Dolycoris baccarum (L.), and Piezodorus lituratus (F.) (Bashan and Cakmak, 2005).

The sugarbeet root maggot, Tetranychus myopaeformis (Röder), is a significant economic pest in the sugarbeet growing areas of North Dakota and Minnesota, USA. This insect overwinters as a mature third-instar larva in the soil at depths of 20 to 30 cm. In addition to being freeze tolerant (Whitfield and Grace, 1985), T. myopaeformis larvae also have the remarkable ability to survive more than 5 years of laboratory storage by examining respiration rates, gene expression, and post-storage survival of T. myopaeformis larvae. They found that most stored larvae were in post-diapause quiescence, whereas very few larvae were in a state of prolonged diapause.

In the present study, internal lipids of long-term stored larvae were compared with those of field-collected diapausing larvae to determine energy costs associated with prolonged storage and to characterize lipid usage patterns during the storage period. In addition, fatty acid composition was assessed in diapausing and stored larvae (subjected to storage periods of 1, 2, 3, and 5 years) to determine if long-term storage impacts the distribution or composition of fatty acids in T. myopaeformis larvae.

2. Material and methods

2.1. Insects and cold storage

All T. myopaeformis larvae used for lipid studies were collected from sugarbeet fields near St. Thomas (Pembina County), North Dakota, USA. Diapausing larvae used for lipid extractions were collected after crop harvest in the months of October to December of 2004 and 2007. Larvae used for total internal lipid studies had been in storage for 1, 2, 3, and 5 years, and were collected in July and August of 2004 and 2007. Larvae used for total internal lipid studies had been in storage for 1, 2, 3, and 5 years, and were collected in July and August of 2004 and 2007. Larvae were frozen immediately following puncturing by adding small quantities of liquid nitrogen to each tube. The tubes were then placed into glass lyophilization flasks, and each flask was connected to a vacuum-operated lyophilizer (Virtis, Gardiner, New York, NY, USA). After lyophilization, dried samples were reweighed in the glass tubes to determine dry weights. Sample size ranged from five to 12 larvae per group. Dried larvae were subsequently suspended in chloroform (CHCl₃) and homogenized in a glass tissue grinder. Internal lipids were obtained by adding more CHCl₃ and filtering through a glass wool plug within a chamagne funnel. Filtrate and solvent rinses were evaporated by heating under a stream of nitrogen gas, and lipid residues were re-dissolved in known volumes of CHCl₃.

Fresh weights were taken on larvae after thoroughly blot-drying them with tissue paper and allowing an additional air drying period of two to 5 min to ensure that they were completely dry. In each larval category studied, groups were divided into samples consisting of five to 12 larvae, and two to three samples were used for analyses. For extraction of internal lipids, larvae were rinsed twice for 1 min with hexane and once for 30 s with CHCl₃ to extract cuticular surface lipids. To extract and recover internal lipids, hexane- and CHCl₃-rinsed larvae were suspended in CHCl₃ and homogenized in a glass tissue grinder, and the resulting tissues were filtered through glass wool using the previously described procedures.

To measure total lipid concentration for each larva, lipids from the pooled larval sample were initially dissolved in 1000–2000 µL of CHCl₃. Twenty five microliters of this mixture was then transferred into pre-weighed aluminum foil boats. Each boat was also weighed after drying, and total lipid weight per larva was calculated as: (lipid wt./25 µL of solvent) × total solvent vol./number of larvae. Total lipids, as a percent of larval wet and dry weights, were calculated using the following formula: (total lipid wt./larva)/avg. wet or dry larval wt. × 100.

2.3. Thin-layer and open-column chromatography

To identify specific lipid classes, extracted and filtered internal lipids were subjected to thin-layer chromatography (TLC) as described by Nelson et al. (2003). Approximately 5 µL of lipid extracts (in CHCl₃) were carefully spotted onto 10×10 cm high performance silica gel plates (EM Science, Gibbstown, NJ, USA) at 1 cm above the bottom edge of the plate along with the following standards: hydrocarbons, wax esters, acetate esters, triacylglycerols (TAGs), fatty acids, and diacylglycerols. Sample lipid extracts and standards were spotted in lanes adjacent to each other, and the plates were placed in a covered glass tank containing hexane/diethyl ether/formic acid (80:20:1 v/v/v) as a developing solvent. The separation and location of lipid bands were visualized by spraying the plates with a solution of 5% sulfuric acid dissolved in 95% ethanol, and charring at 160 °C for 20 min.

Sample lipid extracts were subjected to fractionation by passing them through a 0.5-mm×9.5-cm column of silica gel (Universal Scientific Inc., Atlanta, GA, USA) in a champagne funnel (Nelson et al., 2003). The silica column was cleaned and equilibrated by washing with 8–10 mL of CHCl₃ and 40 mL of hexane. Internal lipids were dissolved in known volumes of hexane and a portion of the sample was applied to the column. An additional 8 mL of hexane, followed by 8 mL of 5% diethyl ether in hexane was added to remove non-polar surface lipid contaminants (hydrocarbons, wax esters, etc.). To remove internal lipids, including the TAGs, the column fraction was eluted with sequential additions of 8 mL of diethyl ether in hexane: 10%, 25%, 50%, 75% and 100%. Column fractions were then analyzed for lipid class content and purity by TLC as described above.
2.4. Hydrolysis and formation of fatty acid derivatives

Triacylglycerol fractions from silica columns were subjected to alkaline hydrolysis to obtain free fatty acids associated with TAGs (Buckner and Hagen, 2003). The eluted TAG fraction of each sample and its associated solvent were transferred into a 1-mL Reacti-Vial® (Thermal Fisher Scientific Inc., Rockford, IL, USA), and the solvent was evaporated over a hot sand bath using nitrogen gas. Forty microliters of benzene were added to each vial and mixed thoroughly. Subsequently, 760 µL of a 5% KOH in methanol solution was added to the mixture and sonicated for 2 min. The vials were then sealed and placed in an aluminum block heated to 75 °C for 3 h. After cooling, the alkaline mixture was neutralized to pH 6–8 by adding 1 N hydrochloric acid. After neutralization, 100–200 µL of CHCl₃ was added to the mixture, followed by 200 µL of water. After vigorous mixing, the lower clear CHCl₃ layer was transferred with a pipette into a new vial. The process was repeated three times by adding CHCl₃ and collecting the clear layers. Small quantities of water in the collected fractions were removed by adding anhydrous magnesium sulfate, shaking the mixture, and allowing it to stand for 20 min. The content of each vial was transferred and filtered using a champagne funnel plugged with a small piece of tissue paper, and the funnel was rinsed with CHCl₃ after filtering.

Fatty acids (from alkaline hydrolysis) were initially derivatized as their trimethylsilyl esters before identification or quantification (Buckner and Hagen, 2003). This procedure first involved adding 20 µL of benzene and 20 µL of BSA (N-O-bis(trimethylsilyl)-acetamide) to the free fatty acid fractions obtained from alkaline hydrolysis, followed by 60 µL of dimethyl formamide. Vial contents were then mixed thoroughly and heated to 75 °C for 20 min. After heating, the samples were cooled to room temperature and analyzed by GC–MS.

Identification of double-bond positions in the monounsaturated fatty acids was carried out by converting the hydrolyzed fatty acids into fatty acid methyl esters (FAMEs) and treating them with dimethyl disulfide (DMDS) (Scribe et al., 1988). The TAG fatty acids were converted to FAMEs using the methods of Buckner and Hagen (2003). Portions of fatty acids within 1-mL Reacti-Vials were mixed with 40 µL of benzene and 400 µL of 10% HCl in methanol. Sample vials were then tightly capped and heated at 75 °C for 40 min. After cooling, 400 µL of water and 400 µL of CHCl₃ were added by mixing. The lower layer (CHCl₃) was removed and transferred into a new vial. The remaining water layer was extracted four times with additional CHCl₃. Anhydrous magnesium sulfate was added to the combined CHCl₃ extracts to remove residual water. The sample solution was then filtered through tissue paper and CHCl₃ was evaporated under a stream of nitrogen gas.

For DMDS derivatization, FAMEs were mixed with a combination of 100 µL of hexane, 100 µL of DMDS, and a 10-µL iodine solution (i.e., 60 mg of I₂ in 1 mL of diethyl ether). The mixture was heated overnight at 72–75 °C in a tightly capped vial. After the mixture had cooled to room temperature, 200 µL of 5% solution of sodium thiosulfate in water was added to absorb the remaining iodine. The aqueous layer was then extracted several times with hexane. Residual water in the combined hexane extracts was removed by adding anhydrous magnesium sulfate. The hexane extracts were passed through filter paper, the solvent evaporated and stored under argon at −20 °C.

2.5. Gas chromatography–mass spectrometry

Fatty acid analyses were carried out by injecting the intact TAG fraction groups from HPLC, trimethylsilyl (TMS) derivatives of TAG fatty acids, and DMDS-treated FAME samples into a Hewlett-Packard (Model 5890A) gas chromatograph equipped with an auto-injector and a cool on-column injection port. Initial column temperature was maintained at 150 °C. The oven was heated to 320 °C and held at that temperature for 20 to 120 min (Nelson, 2001). The injection port was connected to a 12-m × 0.2-mm capillary column (HP Ultra) by a 1-m fused-silica retention gap. The chromatograph was connected to a HP 5970B quadrupole mass-selective detector with the multiplier set at 70 eV, transfer line at 300 °C, and helium as the carrier gas. The column was held at different temperature regimes for the following groups: 1) intact TAG fractions were initially held at 150 °C then temperature was increased at 4 °C/min to 325 °C; 2) TMS derivatives of TAG fatty acids were initially held at 100 °C with temperature subsequently increased at 4 °C/min to 320 °C; and 3) DMDS-treated FAME samples initially maintained at 150 °C, then heated to 320 °C at intervals of 3 to 4 °C per min, and held at 320 °C for 20–40 min. Fatty acid identities and quantities were then confirmed and calculated by comparing retention times with authentic standards.

2.6. High performance liquid chromatography

Intact TAGs were separated and detected by HPLC using a Waters Model 2695 Separations Module (Waters Corp., Milford, MA) equipped with a 250 × 4.6 mm Chromspher 5 Lipid column (5 µm silica derivatized with a cation exchange resin in the Ag⁺ ionic form; Varian, Lake Forest, CA, USA) and a Sedex (Model 55) evaporative light scattering detector (ELSD) (Sedere, Alfortville, France) operated at 28 °C and N₂ (g) pressure at 2.1 bar. The column was equilibrated at 1 mL/min and 30 °C with hexane/toluene/ethyl acetate (90:5:5). TAG fractions from Biosil column chromatography were dissolved in the equilibrating solvent and 5 µL portions injected onto the HPLC column at a flow rate of 1 mL/min. The TAG components were eluted from the column with the following linear solvent gradients: increasing from 0 to 15% acetone in 15 min; and to 100% acetone from 15 to 25 min. For GC–MS analyses, three regions of eluting TAG components from the HPLC column were collected: Group 1 at 9–11 min; Group 2 at 16–19 min; and Group 3 at 20–22 min. All solvents were dried over molecular sieves before use.

3. Results

3.1. Wet and dry larval weights and quantity of total internal lipids

Changes in larval weight were determined for _T. myopaeformis_ during cold storage. Mean wet weight per larva ranged between 17.8 and 21.8 mg in freshly field-collected diapausing larvae and 1-, 2-, 3-, and 5-year stored larvae (Table 1). Although larvae appeared to lose weight as storage period length increased from one to five years, no significant differences in wet weights were observed among larval groups. In contrast, dry weights of diapausing and 1-year stored larvae were significantly (P < 0.0001) higher than 3-year stored larvae, but surprisingly not significantly different from 2- or 5-year stored larvae (Table 1). The quantity of internal lipid, as a percent of wet and dry weights, was highest (i.e., 21.8% and 29.8% of wet and dry weight, respectively) in diapausing larvae, and appeared to decrease progressively with increasing storage time (Fig. 1). In 5-year stored larvae, total internal lipids (TIL) decreased by approximately 78 and

<table>
<thead>
<tr>
<th>Storage status</th>
<th>Diapause n = 3</th>
<th>1 year n = 3</th>
<th>2 years n = 3</th>
<th>3 years n = 3</th>
<th>5 years n = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet mass (mg)</td>
<td>19.56 ± a</td>
<td>21.81 ± a</td>
<td>19.20 ± a</td>
<td>18.60 ± a</td>
<td>17.81 ± a</td>
</tr>
<tr>
<td>Dry mass (mg)</td>
<td>14.31 ± a</td>
<td>14.67 ± a</td>
<td>10.43 ab</td>
<td>8.50 b</td>
<td>9.88 ab</td>
</tr>
</tbody>
</table>

^n = number of samples (5 larvae each). Mean mg values within a row that have the same lower-case letter are not significantly different using a one-way ANOVA (P < 0.0001).
70% when compared to diapausing larvae on wet and dry weight bases, respectively. Exponential curves of percentage TIL, based on wet and dry weights, appear to be parallel. This suggests that there was no water loss in *T. myopaeformis* larvae throughout five years of storage (Fig. 1).

### 3.2. Characterization of fatty acids from triacylglycerols

Lipid class identification by thin-layer chromatography revealed that TAGs were the most predominant class of internal lipids in field-collected diapausing and long-term stored *T. myopaeformis* larvae (data not shown), and there was no apparent change in lipid composition with years of storage. Trace amounts of diacylglycerols and hydrocarbons also were observed in diapausing and stored larvae. Ten prominent fatty acids from TAG fractions were identified and quantified. For the fatty acids from diapausing and 1-, 2-, and 3-year stored larvae, the fractional distributions were similar (Fig. 2). The two major fatty acids were identified as the C16 and C14 monounsaturated fatty acids (16:1 and 14:1, respectively). Lesser amounts of 12:1, 18:1, and 20:1 monounsaturated fatty acids were also present. TMS derivatives of fatty acids from the diapausing and stored larval groups TAG fractions also included the saturated fatty acid, were also detected. TMS derivatives of fatty acids from the diapausing and stored larval groups TAG fractions also included the saturated fatty acids myristic (14:0) and palmitic (16:0), with lesser amounts of lauric (12:0) and stearic (18:0) also present.

#### 3.3. HPLC and GC–MS identification of triacylglycerols

The identifications and distributions of intact TAGs for diapausing larvae were determined and compared to those of 2-year stored larvae. HPLC-ELSD chromatograms showed similar peak profiles for intact TAGs from diapausing and cold-stored larvae. As shown in Fig. 3A, a typical chromatogram of TAGs from 2-year stored larvae consisted of three groups of peaks which eluted in the order of degree of unsaturation as described previously (Buckner et al., 2004). The first group (Group 1) of peaks contained mixtures of TAGs, each with one monounsaturated fatty acid moiety and two saturated fatty acid moieties. Similarly, Group 2 and Group 3 consisted of mixtures of TAGs, each with two and three monounsaturated fatty acid constituents, respectively. Following fractionation by HPLC and collection of column effluent for Groups 1 to 3, portions of each collected group of TAGs from diapausing and 2-year stored larvae were analyzed by GC–MS (Fig. 2). The majority of TAG fatty acids in diapausing and all stored larval groups were monounsaturated (73–78%) and the major constituent, hexadecenoic acid (16:1), comprised 40–50% of the total unsaturated fatty acid composition (Table 2). The double-bond analyses of TAG fatty acids as the DMDS derivatives by GC–MS revealed that the double-bond position for the 16:1 fatty acid was between carbon 9 and 10 and thus identified as palmitoleic acid (9-hexadecenoic acid). For the three 14:1 fatty acid positional isomers, the major positional isomer was 9-tetradecenoic acid (59% of the 14:1 isomers), and lesser distribution amounts of 7-tetradecenoic acid (38%) and 5-tetradecenoic acid (3%). The 14:1 acids made up 21% of the fatty acid composition of diapausing larvae. The 14:1 acids decreased to 13–16.5% in 1- and 2-year stored larvae, respectively, and were highest (28%) in 5-year stored larvae. The 18:1 fatty acid was identified as oleic acid (9-octadecenoic acid) (Table 2). The double bond was located in the Δ9 position for the 12:1 fatty acid (5-dodecenoic acid). Small amounts of the 20:1 fatty acid, 11-eicosenoic acid, were also detected. TMS derivatives of fatty acids from the diapausing and stored larval groups TAG fractions also included the saturated fatty acids myristic (14:0) and palmitic (16:0), with lesser amounts of lauric (12:0) and stearic (18:0) also present.

#### Table 2 Composition of triacylglycerol fatty acids in diapausing and laboratory stored *T. myopaeformis* larvae.

<table>
<thead>
<tr>
<th>Fatty acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Common name</th>
<th>Scientific name</th>
<th>% Composition&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Diapause</th>
<th>Storage period (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Lauroleic (Lo)</td>
<td>5-Laurolesic acid</td>
<td>5-Dodecenoic acid</td>
<td>5.2</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Lauric (La)</td>
<td>Dodecenoic acid</td>
<td>2.9</td>
<td>2.7</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Myristoleic (Mo)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9-Tetradecenoic acid</td>
<td>21.0</td>
<td>13.4</td>
<td>16.5</td>
<td>28.3</td>
</tr>
<tr>
<td>Myristic (M)</td>
<td>Tetradeconoic acid</td>
<td>8.0</td>
<td>10.3</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Palmitoleic (Po)</td>
<td>9-Hexadecenoic acid</td>
<td>48.0</td>
<td>49.9</td>
<td>49.7</td>
<td>41.6</td>
</tr>
<tr>
<td>Palmitic (P)</td>
<td>Hexadecenoic acid</td>
<td>7.5</td>
<td>10.1</td>
<td>10.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Oleic (O)</td>
<td>9-Octadecenoic acid</td>
<td>3.6</td>
<td>6.1</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Stearic (S)</td>
<td>Octadecenoic acid</td>
<td>0.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Gondoic (Go)</td>
<td>11-Eicosenoic acid</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
<td>t</td>
</tr>
<tr>
<td>Arachidic (A)</td>
<td>Eicosanoic acid</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers to the left and the right of the colon refer to the number of carbons and the number of double bonds, respectively, and the Δ numbers indicate the carbon positions for the double bond(s), counting from the carboxyl end.

<sup>b</sup> Percentage composition values were calculated from quantities of fatty acid TMS derivatives, as determined from the GC–MS integrated peak area for each component and standard curves for authentic standards. <i>t</i> = trace amount (<0.1).

<sup>c</sup> Double bond positions were identified by GC–MS of DMDS-treated FAMES.

<sup>d</sup> The major 14:1 double bond positional isomer was 9-tetradecenoic acid (59%) with lesser amounts of 7-tetradecenoic acid (38%) and 5-tetradecenoic acid (3%).
larval samples showed similar distributions of mainly even-carbon chain lengths of 44, 46, and 48 were predominant in all the three eluted groups, followed by C50 and C52 (Table 3). Trace amounts of C40 and C54 TAGs, which were not visible in HPLC chromatograms, were found in both larval test groups (Fig. 3A). The percent composition of individual TAGs among the three eluted groups remained similar in both larval groups (Table 3).

The identity of individual fatty acid constituents of TAGs was derived from GC–MS analyses. TAG component mixtures were structurally identified by interpretation of their electron ionization mass spectra. Diagnostic ions included the molecular ion, the diacylglyceride ions (with the most intense ion corresponding to the loss of one unsaturated fatty acid), and the monoglyceride ions and acyl ions for each fatty acid constituent. Many of the peaks in the TAG fraction contained one or more TAGs and a single mass spectrum contained diagnostic ions for more than one TAG (Fig. 3B). As shown in Table 4, individual TAGs were listed and arranged in ascending order of chain length and unsaturation. For the Group 1 TAGs, 16 C40–C52 components and positional isomers were structurally identified. Group 2 consisted of 16 C42–C52 TAG components, and 12 C40–C52 components were identified in the Group 3 fractions. Consistent with composition data for fatty acids from TAGs (Table 2), and as shown in Table 4 for the structures of intact TAGs, the major fatty acid constituent was palmitoleic acid (Po), which was identified in more than 35 TAG compounds. The second most abundant fatty acid, myristoleic acid (Mo), was identified in 12 TAG compounds.

From GC–MS analyses of HPLC-resolved Groups 2 and 3, the major C40 TAG components were identified as myristoleoyl palmitoyl palmitoleoyl glycerol (MoPoPo) and myristoyl palmitoleoyl palmitoleoyl glycerol (MoPoPo), respectively (Table 4). The major C48 components of Groups 2 and 3 TAGs were identified as palmitoyl palmitoleoyl palmitoleoyl glycerol (PPoPo) and palmitoleoyl palmitoleoyl palmitoleoyl glycerol (PoPoPo), respectively. In Group 1 TAGs (those with one monounsaturated fatty acid moiety), the only C48 component was myristoyl palmitoyl palmitoleoyl glycerol (MPPo), and the major C44 component was palmitoyl palmitoyl palmitoleoyl glycerol (PPPo). Mass spectra data also identified the presence of the C44 positional isomers MPoPS and MPSo in Group 1, and for Group 2, the positional isomers for C42 and C44 TAGs (MMPo, MoMPPo, and MoPoPPo, respectively) (Table 4).

4. Discussion

Insects in temperate regions often use diapause, quiescence, and metamorphosis to endure major survival challenges associated with harsh climatic periods or seasons when resources are limited (Tauber et al., 1986; Danks, 1987). Although diapausing and quiescent insects share a common feature of maintaining low metabolic activity, the cost of extended dormancy periods can draw heavily on energy reserves (Ellers and van Alphen, 2002). Lipids typically make up less than 10% of the total wet body weight in most insect species (Fast,
The fact that total internal lipids were highest in diapausing T. myopaeformis larvae and gradually decreased with increased storage time suggests that lipids are important energy sources in this species (Fig. 1). Lipids have been shown to range between 1.5 and 5% fresh body weight in different stages of the silverleaf whitefly, Bemisia argentifolii Bellows and Perring (Buckner and Hagen, 2003), 5.9 and 8.6% in larval, pupal, and adult honeybees, Apis mellifera L. (Robinson and Nation, 1970), 9% in diapausing flesh fly, Sarcophaga crassipalpis Macquart pupae (Adedokun and Denlinger, 1985), and 6.5% in diapausing 3rd-instar larvae of the burnet moth Zygaena trifolii Esper (Wipking et al., 1995). In contrast, Lambremont et al. (1964) reported 18 to 25% of total body fat in diapausing adult boll weevils, Anthonomus grandis Boheman. Similarly, Buckner et al. (2004) showed that overwintering prepupae of the alfalfa leafcutting bee, M. rotundata (F.), possessed copious amounts (i.e., 20% of the wet weight) of stored lipids. Diapausing 3rd-instar sugarbeet root maggot larvae can also be included in the latter category of the above-mentioned insects, as lipids made up 21.8% (wet-weight basis) of larval body weight in our study (Fig. 1).

Table 4
Identified 58Rm triacylglycerols and their fatty acid constituents.

<table>
<thead>
<tr>
<th>Carbon number</th>
<th>Triacylglycerols and fatty acid constituents</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Double bond</td>
<td>C40</td>
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*a Number of total carbons in fatty acids of triacylglycerols that were separated into three groups by HPLC and structurally identified by GC-MS.

*b The most prominent positional TAG isomer is listed first for each of the TAG chain lengths. Abbreviations for common names of fatty acid constituents were as de
c

**c The position of fatty acid constituents for positional isomer triacylglycerols was based on interpretation and abundance of diagnostic monoglyceride and diglyceride mass spectral fragment ions.
In conclusion, term laboratory cold storage (post-diapause quiescence). Pattern over time to sustain themselves through diapause and long-term stored larvae revealed that there were no continued through the two groups, except for the gradual decrease in the quantity of fatty acids between diapause and long-term stored larvae. High percentages of unsaturated fatty acids have been reported for the overwintering prepupae of the alfalfa leaf weevil, (Ohtsu et al., 1993; Joanisse and Storey, 1996; Bennett et al., 1997).

The TAG profile of the 2-year stored sugarcane root maggots was nearly identical to that of the diapauing larvae (data not shown). Similar resemblance was also identified in their respiration rates (Chirumamilla et al., 2008).

The results of this study showed that gradual lipid consumption continued through five years of cold storage in T. myopaeformis larvae. The quantitative and qualitative analyses of fatty acid profiles in diapauing and long-term stored larvae revealed that there were no differences in the composition or distribution of fatty acids between the two groups, except for the gradual decrease in the quantity of individual fatty acids between diapause and five years of cold storage. In conclusion, T. myopaeformis larvae are using lipids in a gradual pattern over time to sustain themselves through diapause and long-term laboratory cold storage (post-diapause quiescence).

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References


