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***Orijinal araştırma (Original article)***

# Seasonal changes in fatty acid composition of *Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) (Heteroptera: Pentatomidae) adults

Özlem ÇAKMAK[1]*

## Summary

The goal of the study was to investigate the role of phospholipid and triacylglycerol fatty acid compositional changes in *Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) (Heteroptera: Pentatomidae) with respect to seasonal changes. *E. inconspicuous* adults were collected from Diyarbakır, Turkey in 2007-2008. The fatty acid compositions of phospholipid and triacylglycerol fractions that were extracted from whole-body of adult *E. inconspicuous* were isolated and analyzed using gas chromatography and gas chromatography-mass spectrometry. Qualitative analysis has revealed the presence of 15 fatty acids during most of the months. The major components were C16 and C18 saturated and unsaturated components which are ubiquitous in most animal species. In addition to these components, three odd-chain (C13:0), (C15:0), (C17:0), and prostaglandin precursor fatty acids were found. The fatty acid profiles of phospholipids and triacylglycerols have some diferences. In contrast to triacylglycerol fraction, linolenic acid and C20 polyunsaturated fatty acids increased during autumn and winter in phospholipid fraction were detected. The unsaturated fatty acid to saturated fatty acid ratio significantly increased in both fractions but the increase was dramatic in phospholipid fraction during autumn, and reaches its maximum level in january and february, when outdoor temperatures are low. Thus, temperature seems to play an important role in seasonal variation of lipid metabolism of *E. inconspicuous*. Preventing cellular damage due to low temperatures is a major challenge for insects. These findings indicate that *E. inconspicuous* can modify its fatty acid composition in response to changes in environmental conditions.

**Key words:** *Eysarcoris inconspicuous*, phospholipid, triacylglycerol, fatty acid, seasonal change

**Anahtar sözcükler:** *Eysarcoris inconspicuous*, fosfolipit, triaçilgliserol, yağ asidi, mevsimsel değişim

[1] Department of Biology, Education Faculty, Dicle University, 21280 Diyarbakır, Turkey
* Sorumlu yazar (Corresponding author) e-mail: ocakmak@dicle.edu.tr
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## Introduction

Insects are the most diverse fauna on the earth. Many overwintering insects inhabiting the temperate zones endure environmental stresses by entering diapause and developing cold-hardiness (Denlinger, 1991). During diapause, direct development (morphogenesis) is endogenously arrested and an alternative program of physiological events proceeds, which is significantly modulated by changing environmental conditions (Khani et al., 2007). As temperatures decrease, cellular membranes with a static composition tend to increase rigidity until regions of the membrane transition from a liquid crystal to gel state and the membrane loses its ability to function as a barrier (Cossins, 1983). To counter this effect, the membrane may change in composition to maintain the liquid crystalline state at lower temperatures, a process known as homeoviscous adaptation (Sinensky, 1974; Candan & Suludere, 2001.). The best evidence for homeoviscous adaption is determined directly by the measurement of membrane viscosity at fluctuating temperatures. Furthermore, it can also be inferred from compositional changes in the membrane. Such changes take place at low temperatures with an increase in points of unsaturation along phospholipid (PL) fatty acid (FA) chains. Moreover, it may manifest itself in the form changes in other membrane composition characteristics such as increased cholesterol content or a change in PL class distribution (Hazel, 1995). The protection from low temperature that diapause and rapid cold hardening imparts to the insect may be positively influenced by changes in membrane composition. Diapause-induced alteration in membrane PLs has been demonstrated for several insect species in which the diapause program also features cold hardiness (Hodková et al., 1999; Kostal et al., 2003; Bashan & Cakmak, 2005; Michaud & Delinger, 2006).

*Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) (Heteroptera: Pentatomidae) is one of the most important pests of wheat, rice, and raspberry in Turkey. The insects complete their diapause stage under stones and in refuge plants, such as *Arctostaphylos uva-ursi* (L.) (Ericales: Ericaceae), *Avena elatior* (L.) (Poales: Poaceae), and *Juniperus communis* (L.) (Pinales: Cupressaceae) in the mountains. When the surrounding temperature reaches to 18°C, in early may, they migrate from overwintering localities to cereal areas, and cause damage feeding on cereal throughout the development of plants. Following the landing, during a period of 15-20 days, they feed and mate, and then start to lay eggs, and die. The nymphs hatch from eggs and become new-generation adults after a period of 25-30 days. After the harvest, as the weather starts cooling, these new-generation adults migrate to overwintering areas. Once the weather gets cold, their activities terminate and they spend winter in diapause mode in these areas. In the study, we investigate the role of PL and triacylglycerol (TG) FA compositional changes in *E. inconspicuous* as a result of seasonal changes.

## Materials and Methods

### Biological specimens

Prediapausing adults of *E. inconspicuous* were collected with nets and light traps from Diyarbakır, Turkey (37° 54'N, 40° 14'E; at an altitude of about 850 m) and diapausing adults were collected from Karacadag mountain (37° 59'N, 40° 12'E; at an altitude of about 1600 m). The insects were not collected in may and june months, because the insects live egg and first nymhal stage of their life cycle in these months.

### Fatty acids analysis

The insects were processed for lipid extraction and analysis following the methods described by Blingh & Dyer (1959). For insect analysis, three groups of 25 adults (total weight ~1.4 g) were used because of their low individual weights. Each sample was replicated three times. Insects were homogenized in glass tubes and extracted three times with chloroform/methanol (2:1, v/v). Autoxidation of unsaturated components was minimized by adding 50 μl of 2% butylated hydroxytoluene in chloroform to each sample during the extraction process.

The total lipid extracts were dried under a stream of $N_2$. Then PL and TG fractions were isolated by thin-layer chromatography (TLC), using Silica Gel G TLC plates (20 by 20 cm, 0.25 mm thick). After applying the total lipid extracts, the TLC plates were developed in petroleum ether: diethyl ether: acetic acid (80:20:1, v/v). Lipid fractions were made visible by spraying the TLC plates with 2',7'-dichlorofluorescein (Supelco, Supelco Park, PA, USA), and PL and TG fractions were identified by corresponding standards.

The PL and TG fractions were scraped into reaction vials, and the associated FAs were transmethylated by refluxing the fractions in acidified methanol for 90 min at 85°C. The fatty acid methyl esters (FAMEs) were extracted from the reaction vials three times with hexane, and then they were concentrated.

### Gas chromatography

The FAMEs were analyzed by gas chromatography using a Ati Unicam 610 gas chromatograph equipped with a SP-2330 capillary column (30 m by 0.25 mm i.d., 0.2 μm film thickness, Supelco), a flame ionization detector, and a Unicam 4815 recording integrator. A split injection of 0.5 μl was used. The temperature condition detector was 250°C. The oven temperature was kept at 180°C for 5 min, then reached to 200°C with a ramp rate of 2°C /min, and then was kept at this temperature for 15 min. FAMEs were identified by comparisons of retention times with authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Individual FAMEs were identified by comparisons with the chromatographic behaviors of authentic standards.

### Gas chromatography-chemical ionization mass spectrometry

The chemical structures of the FAMEs were confirmed by capillary gas chromatography-mass spectrometry (GC-MS) (HP 5890-E series GC-System, Hewlett-Packard, Palo Alto, CA, USA) with mass-selective detection. An Innowax column (30m by 0.25 mm i.d., 0.25 µm film thickness) was used, and the temperature was increased gradually from 150 to 230$^{o}$C at a 2$^{o}$C/min increase with an initial hold of 6 min. The carrier gas was helium (1 mL/min) and the split ratio was 1:50. The injection port and the detector temperatures were 250$^{o}$C and 300$^{o}$C, respectively. The mass spectrometer was operated in the electron impact ionization mode (70 eV). Chemical structures of the FAMEs were determined by comparison of the spectra with the Wiley 275 and Nist 98 databank, and by comparing obtained spectra with that of authentic standards.

### Weather data

The average of the whole day air temperatures (Table 1) were recorded by a weather station (Turkish State Meteorological Service) from the sampling sites.

Table 1. The average of the whole day air temperatures in 2007-2008 in Diyarbakır, Turkey

| Months | Average air temperature ($^{o}$C) |
|---|---|
| July | 37,1 |
| August | 36,3 |
| September | 30,6 |
| October | 20,9 |
| November | 11,9 |
| December | 3,8 |
| January | 2,0 |
| February | 5,5 |
| March | 9,2 |
| April | 14,8 |

### Statistical analysis

The analysis was performed using a commercial statistical software program (SPSS 13.0). Statistical analysis of percentages of FA were tested by analysis of variance (ANOVA), and comparisons between means were performed with Tukey test. Differences between means were evaluated as significant when $P<0.05$. For investigating of a supposed linear relationship between two variables, Pearson's correlation test was used.

## Results

Seasonal changes of the fatty acid compositions percentages of PL which were prepared from whole-body of adult *E. inconspicuous* in different months are presented in Table 2. Additionally, saturated, monounsaturated, and polyunsaturated fatty acids percentages of PL are shown in Figure 1.

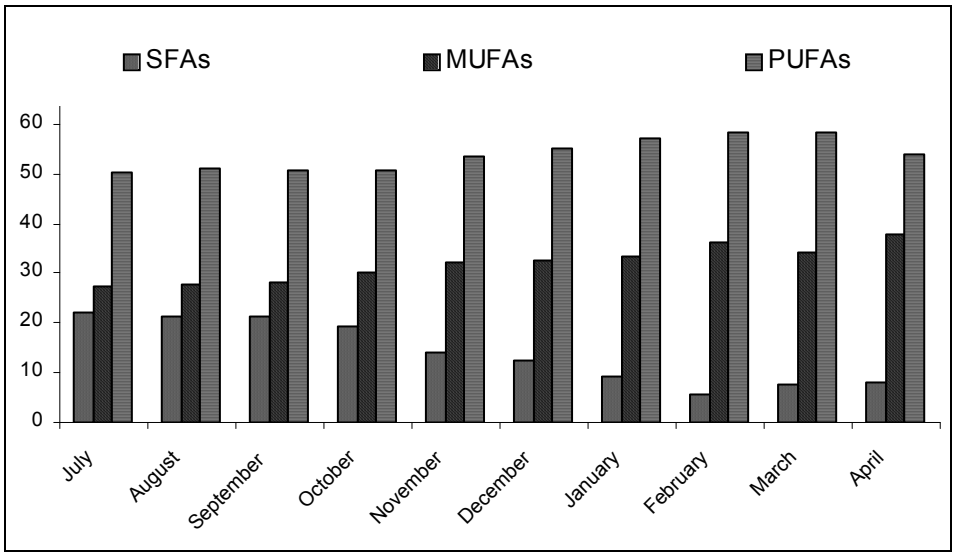


Figure 1. Saturated, monounsaturated and polyunsaturated fatty acids percentages of *Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) in phospholipid fraction.

Predominant FA components of PL were C16:0 (palmitic acid), C18:0 (stearic acid), C18:1n-9 (oleic acid), and C18:2n-6 (linoleic acid), representing over 75% of the FAs, in which C18:1n-9 was most abundant (~22-31% in PL and 29-40% in TG fractions). Among minor components which constitude less than 4% of FAs, C15:0 (pentadecanoic acid), C17:0 (heptadecanoic acid), C20:4n-6 (arachidonic acid) and C20:5n-3 (eicosapentaenoic acid) were barely detected.

Seasonal changes of the fatty acid compositions percentages of TG which were prepared from whole-body of adult *E. inconspicuous* in different months are presented in Table 3. Additionally, saturated, monounsaturated, and polyunsaturated fatty acids percentages of TG are shown in Figure 2.

In comparison to PLs, the FA profiles of TGs which were prepared from *E. inconspicuous* had higher proportions of C16:0, and lower proportions of two polyunsaturated fatty acids (PUFAs), C18:2n-6 and C18:3n-3 (linolenic acid). Palmitic acid constituted comprised about 3-11% of PLs and about 19-29% of TGs. Linoleic acid accounted for made up about 27-31% of PLs and 10-18% of TGs. Linolenic acid comprised about 11-13% of PLs and 2-6% of TGs.

Table 2. Fatty acid compositions, as proportions of total fatty acids, in phospholipid prepared from total lipid extract of whole *Eysarcoris inconspicuous* (Herrich- Schaffer, 1844) in different months

| Fatty acids | July (mean◊ ±S.D)# | August (mean◊ ±S.D)# | September (mean◊ ±S.D)# | October (mean◊ ±S.D)# | November (mean◊ ±S.D)# | December (mean◊ ±S.D)# | January (mean◊ ±S.D)# | February (mean◊ ±S.D)# | March (mean◊ ±S.D)# | April (mean◊ ±S.D)# |
|---|---|---|---|---|---|---|---|---|---|---|
| C13:0 | 0.08 ± 0.02a | - | - | - | - | - | - | - | - | 0.10 ± 0.01a |
| C14:0 | 0.21 ± 0.02a | 0.13 ± 0.01b | 0.16 ± 0.02c | 0.17 ± 0.02c | 0.17 ± 0.01c | - | - | - | - | 0.23 ± 0.02a |
| C15:0 | 0.06 ± 0.03a | 0.08 ± 0.02a | 0.11 ± 0.03b | 0.09 ± 0.02a | 0.09 ± 0.03a | 0.05 ± 0.01c | 0.04 ± 0.01d | 0.03 ± 0.01d | 0.05 ± 0.02c | 0.03 ± 0.01d |
| C16:0 | 10.73 ± 0.22a | 10.45 ± 0.18a | 10.35 ± 0.27a | 8.13 ± 0.25b | 8.43 ± 1.02b | 7.29 ± 0.58c | 5.31 ± 1.08d | 3.42 ± 0.19e | 4.37 ± 0.23e | 4.74 ± 0.23e |
| C16:1n-7 | 2.12 ± 0.25a | 2.35 ± 0.15a | 2.72 ± 0.08b | 2.93 ± 0.24b | 2.19 ± 0.14a | 2.56 ± 0.14b | 3.17 ± 0.21b | 4.44 ± 0.20c | 2.02 ± 0.15a | 5.13 ± 0.37d |
| C17:0 | 0.10 ± 0.03a | 0.08 ± 0.02a | 0.09 ± 0.02a | - | - | - | - | - | - | - |
| C18:0 | 10.94 ± 0.10a | 10.63 ± 0.25a | 10.51 ± 0.15a | 10.74 ± 0.70a | 5.44 ± 0.31b | 5.14 ± 0.42b | 4.03 ± 0.32c | 2.19 ± 0.17d | 3.13 ± 0.30e | 3.06 ± 0.11e |
| C18:1n-9 | 23.14 ± 1.45a | 22.32 ± 1.13a | 22.52 ± 0.82a | 25.27 ± 1.02b | 28.33 ± 1.15c | 28.34 ± 0.52c | 29.02 ± 1.11d | 30.15 ± 1.51e | 30.18 ± 1.29e | 30.90 ± 1.20f |
| C18:2n-6 | 31.12 ± 1.52a | 30.30 ±1.52b | 28.50 ± 1.30c | 28.30 ±1.47c | 28.49 ± 0.82c | 29.12 ± 1.65d | 29.01 ± 1.37d | 30.16 ±1.52a | 29.10 ± 1.74d | 27.18 ± 1.59e |
| C18:3n-3 | 11.57 ± 0.52a | 11.50 ± 0.80a | 12.47 ± 0.73b | 12.16 ± 0.91c | 12.31± 0.89b | 12.49 ± 0.82b | 13.36 ± 0.33e | 13.11 ± 0.18e | 13.23 ± 0.12e | 13.45 ± 0.19e |
| C20:1n-9 | 2.18 ± 0.11a | 2.94 ± 0.21a | 2.87 ± 0.13a | 2.13 ± 0.28a | 1.82 ± 0.22a | 1.65 ± 0.16b | 1.11 ± 0.30c | 1.42 ± 0.36d | 2.13 ± 0.55a | 1.91 ± 0.34a |
| C20:2n-6 | 2.14 ± 0.17a | 3.05 ± 0.11b | 3.49 ± 0.12c | 3.31 ± 0.15c | 4.39 ± 0.24d | 4.54 ± 0.18d | 5.20 ± 0.11e | 5.38 ± 0.18e | 5.45 ± 0.15e | 4.67 ± 0.12f |
| C20:3n-6 | 2.08 ± 0.13a | 2.15 ± 0.29a | 2.29 ± 0.15a | 2.84 ± 0.26b | 3.05 ± 0.73b | 3.48 ± 0.70c | 4.10 ± 0.15d | 3.13 ± 0.39b | 4.54 ± 0.45d | 4.30 ± 0.61d |
| C20:4n-6 | 1.37 ± 0.09a | 1.41 ± 0.17a | 1.22 ± 0.14a | 1.15 ± 0.12a | 2.23 ± 0.15b | 2.16 ± 0.20b | 2.41 ± 0.38b | 3.16 ± 0.27c | 2.24 ± 0.53b | 2.18 ± 0.67b |
| C20:5n-3 | 2.16 ± 0.08a | 2.61 ± 0.15b | 2.70 ± 0.05b | 2.78 ± 0.03b | 3.06 ± 0.14c | 3.18 ± 0.11c | 3.24 ± 0.09c | 3.41 ± 0.24d | 3.88 ± 037d | 2.12 ± 0.56a |

◊ Averages of three replicate using 25 adults per replicates.
# Means with the same letter in each row are not significantly different from each other, P>0.05

Table 3. Fatty acid compositions, as proportions of total fatty acids, in triacylglycerol prepared from total lipid extract of whole *Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) in different months

| Fatty acids | July (mean◊ ±S.D)# | August (mean◊ ±S.D)# | September (mean◊ ±S.D)# | October (mean◊ ±S.D)# | November (mean◊ ±S.D)# | December (mean◊ ±S.D)# | January (mean◊ ±S.D)# | February (mean◊ ±S.D)# | March (mean◊ ±S.D)# | April (mean◊ ±S.D)# |
|---|---|---|---|---|---|---|---|---|---|---|
| C13:0 | 1.02 ± 0.08a | - | 0.70 ± 0.04b | - | - | - | - | - | - | - |
| C14:0 | 0.30 ± 0.02a | 0.32 ± 0.01a | 0.38 ± 0.03b | - | - | - | - | - | - | 0.33 ± 0.01a |
| C15:0 | - | 0.13 ± 0.03a | 0.18 ± 0.06b | 0.10 ± 0.04c | 0.09 ± 0.03c | 0.06 ± 0.08d | 0.05 ± 0.08d | 0.09 ± 0.04c | 0.03 ± 0.02e | 0.03 ± 0.01e |
| C16:0 | 28.80 ± 0.30a | 27.42 ± 0.41a | 27.30 ± 0.60a | 25.20 ± 0.25b | 20.93 ± 1.02c | 20.20 ± 0.58c | 19.37 ± 1.08d | 19.02 ± 0.19d | 20.45 ± 0.23c | 20.65 ± 0.23c |
| C16:1n-7 | 7.26 ± 0.25a | 7.30 ± 0.11a | 7.87 ± 0.09a | 7.63 ± 0.28a | 8.12 ± 0.10b | 8.29 ± 0.14b | 9.11 ± 0.21c | 10.94 ± 0.24d | 11.07 ± 0.18d | 10.66 ± 0.37d |
| C17:0 | 0.30 ± 0.09 | - | - | - | - | - | - | - | - | - |
| C18:0 | 12.59 ± 0.13a | 14.06 ± 0.25b | 14.01 ± 0.31b | 13.13 ± 0.43c | 13.04 ± 0.48c | 10.15 ± 0.39d | 9.13 ± 0.30d | 10.13 ± 0.52d | 9.13 ± 0.33d | 10.13 ± 0.41d |
| C18:1n-9 | 30.72 ± 0.76a | 29.01 ± 0.30b | 31.72 ± 0.42c | 31.03 ± 0.02c | 33.03 ± 0.25d | 38.03 ± 0.63e | 40.03 ± 1.23f | 38.05 ± 0.51e | 37.03 ± 0.22e | 36.63 ± 1.22g |
| C18:2n-6 | 12.65 ± 0.79a | 13.35 ± 0.61a | 10.39 ± 0.87b | 14.95 ± 0.40c | 18.41 ± 0.42d | 16.19 ± 0.63e | 16.01 ± 0.22e | 15.06 ± 0.80f | 15.16 ± 0.74f | 13.17 ± 0.52a |
| C18:3n-3 | 4.02 ± 0.11a | 6.50 ± 1.18b | 5.70 ± 0.31c | 5.81 ± 0.33c | 3.82 ± 0.84d | 3.71 ± 0.53d | 2.38 ± 0.33e | 2.19 ± 0.16e | 3.21 ± 0.38f | 3.45 ± 0.25f |
| C20:1n-9 | 1.02 ± 0.14a | 0.94 ± 0.25a | 0.87 ± 0.13a | 1.13 ± 0.28a | 1.28 ± 0.31a | 1.65 ± 0.35b | 2.13 ± 0.77c | 2.46 ± 0.41b | 1.63 ± 0.50b | 1.11 ± 0.21a |
| C20:2n-6 | 0.87 ± 0.17a | 0.55 ± 0.19b | 0.44 ± 0.38c | 0.56 ± 0.15b | 0.39 ± 0.24c | 0.30 ± 0.13d | 0.32 ± 0.18d | 0.18 ± 0.06e | 0.35 ± 0.05d | 0.21 ± 0.02g |
| C20:3n-6 | 0.22 ± 0.13a | 0.16 ± 0.09b | 0.20 ± 0.12a | 0.24 ± 0.20a | 0.65 ± 0.81c | 1.13 ± 0.13d | 1.18 ± 0.10d | 1.23 ± 0.09d | 1.54 ± 0.18e | 1.30 ± 0.32d |
| C20:4n-6 | 0.07 ± 0.03a | 0.10 ± 0.07b | 0.14 ± 0.16c | 0.15 ± 0.11c | 0.18 ± 0.12d | 0.18 ± 0.23d | 0.15 ± 0.09c | 0.19 ± 0.19d | 0.22 ± 0.53e | 0.18 ± 0.30d |
| C20:5n-3 | 0.06 ± 0.03a | 0.06 ± 0.05a | 0.10 ± 0.05b | 0.07 ± 0.04a | 0.06 ± 0.04a | 0.10 ± 0.01b | 0.14 ± 0.04c | 0.11 ± 0.04d | 0.18 ± 0.04e | 0.15 ± 0.04c |

◊ Averages of three replicate using 25 adults per replicates.

# Means with the same letter in each row are not significantly different from each other, P>0.05

Diapausing adults were collected from Karacadag mountain in november, december, january, february, march and april in 2007-2008. In PL fraction, low temperature acclimation caused a decrase in the amounts of C16:0 and C18:0, and an increase in the amounts of C18:1n-9 and C18:3n-3. There was a negative correlation between temperature and the proportion of C18:1n-9 ($r$= -0.80; $P$<0.001), C18:3n-3 ($r$= -0.32; $P$<0.001).

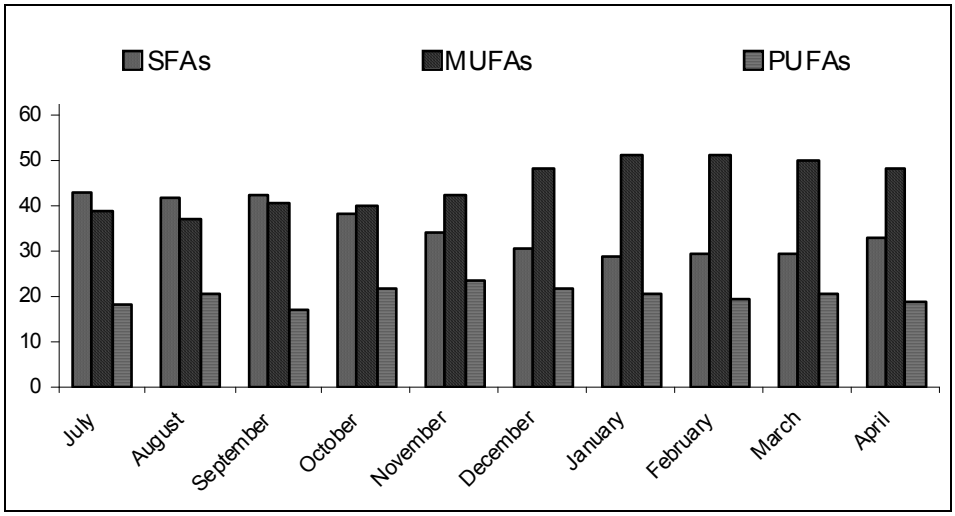


Figure 2. Saturated, monounsaturated and polyunsaturated fatty acids percentage of *Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) in triacylglycerol fraction.

Low temperature acclimation also caused a decrease in the amounts of C16:0, C18:0, C18:3n-3, and increase in the amounts of C16:1n-7 (palmitoleic acid), C18:1n-9 and C18:2n-6 in TG fraction. Palmitic acid was maximum in non–diapause (28.80%), but decreased significantly in november with transition to diapause (20.93%), and then remained constant around 19-20% during autumn and winter seasons. There was a positive correlation ($r$= 0.48; $P$<0.006) between temperature and C16:0. On the other hand, there was a negative correlation between temperature and the proportion of C16:1n-7 ($r$= -0.36; $P$<0.001), C18:1n-9 ($r$= -0.75; $P$<0.001), and C18:2n-6 ($r$= -0.52; $P$<0.001). In addition, the proportion of C20:1n-9 (eicosenoic acid) and C20:3n-6 (eicosatrienoic acid) increased in winter. The unsaturated fatty acids (UFAs) to saturated fatty acids (SFAs) ratio increased in both fractions. The possitive correlation between the proportion of UFA/SFA was stronger in PL fraction ($r$= 0.97) than TG ($r$= 0.42).

## Discussion

The major FA composition of both fractions from whole body of *E. inconspicuous* adults include C16:0, C16:1n-7, C18:0, C18:1n-9 and C18:2n-6,

which have been recorded from Heteroptera and most other insect orders (Spike et al., 1991; Stanley-Samuelson et al., 1988; Hodková et al., 1999; Bashan & Cakmak 2005; Cakmak et al. 2005; 2007; 2008).

Here, we have found differences in FA composition dependent upon seasonality. In PL and TG fractions, SFA amount is significantly higher in summer and autumn than winter and early spring. New-generation adults of *E. inconspicuous* feed on wheat voraciously and store lipid in late spring. Diapausing is induced during late summer and early autumn. During this period short days inhibit reproductive activity and lipid is stored to support the energy demands of insects that hibernate. In particular, diapausing adults had higher proportions of the monounsaturated fatty acids, C16:1n-7, C18:1n-9 and C18:2n-6, and lower proportions of the SFAs, C16:0 and 18:0 in both fractions. In contrast to TG, C18:3n-3 and C20 PUFAs increased in PL fraction during autumn and winter seasons. In PL fraction, the UFA/SFA ratio significantly increased from 3.67 to 6.07 during autumn and from 6.07 to 16.73 during winter, due to the increase of C18:1n-9 and C18:3n-3 at the expense of saturated C16:0 and C18:0. In TG fraction, the UFA/SFA ratio also increased from 1.38 to 1.93 during autumn and from 1.93 to 2.41 during winter, due to the increase of C16:1n-7, C18:1n-9, and C18:2n-6 at the expense of C16:0, C18:0 and 18:3n-3. Oleic acid provides the best environment for critical membrane proteins, such as membrane ATPases. This enzyme functions at optimum levels when oleic acid is present in the cell membrane (Starling et al., 1993). Thus an increase in oleic acid in response to or in preparation for low-temperatures may provide proper fluidity of the membrane without sacrificing the delicate balance needed to keep sensitive membrane proteins maintaining optimum function. *Eurosta solidaginis* (Fitch, 1855) (Diptera: Tephritidae) upregulates oleic acid when it acquires freezing tolerance (Bennett et al., 1997), and two diapausing heteropterans were also found to have increased oleic acid levels during diapause (Bashan & Cakmak, 2005). Oleic acid is energetically more favorable to manufacture than linoleic acid (one less double bond). Hence insects that upregulate oleic acid rather than linoleic acid in preparation for low temperatures may be preserving finite energy reserves while still gaining the benefit of a wide window of fluidity. It is possible that membrane of *E. inconspicuous* are sufficiently fluid so that the increase of unsaturation is needed to prevent deleterious transitions to the gel phase at low temperatures. Oleic acid not only promotes membrane fluidity at low temperatures but also allows the cell membrane to maintain a liquid crystalline state when if temperatures increase (Michaud & Denlinger, 2006). Ohtsu et al. (1998) suggest that the increased of $C_{16}$ fatty acids enlarges the range of temperatures at which membranes are fluid and results in both cold and heat tolerance. Thus, the increase in the proportion of C16:1n-7 acid might be related to both evolutionary and seasonal adaptation to cold in *E. inconspicuous*.

Many studies that examine changes in phospholipids due to cold acclimation or diapause in insects report that 18:2n-6 is the FA that increases for winter climates (Hodkova et al., 1999; Kostal et al., 2003; Overgaard et al., 2005). Only 14 of 40 insects investigated possess $\Delta^{12}$ desaturase, the enzyme needed for adding a double bond to oleic acid to synthesize linoleic acid (Cripps et al., 1986). Insects that do not have a $\Delta^{12}$ desaturase gene or cannot regulate its expression may utilize the $\Delta^{9}$ desaturase instead to initiate membrane FA changes. Both desaturases have been shown to be transcribed and activated at low temperatures (Hsieh & Kuo, 2005). A measurement of the expression and activity levels of these desaturase genes may provide a clear reason for why low-temperature phospholipid changes in certain insects favors one FA over another.

Only in PL fraction of *E. inconspicuous*, the proportion of C18:3n-3, increased in winter. Changes occur during the winter when the insect is not feeding. Hence, it must take place during the transfer of these fatty acids from one lipid fraction to another. It appears that lower linolenic acid in the TG fraction during the winter corresponds to higher levels in the PL fraction. It also makes sense that the most significant changes would be with fatty acids that the insect can biosynthesize, such as oleic acid. As temperatures decrease, cellular membranes with a static composition tend to increase rigidity, until regions of the membrane transition from a liquid crystal to gel state and the membrane loses its ability to function as a barrier (Cossins, 1983). It is possible that membrane of *E. inconspicuous* are sufficiently fluid so that the increase of unsaturation is needed to prevent deleterious transitions to the gel phase at low temperatures. This observation in result from *E. inconspicuous* is in agreement with many studies of insects in diapause status, which involves adaptation having adapted by increasing the ratio of UFA to SFA, during cold acclimatization in autumn and winter (Baldus & Mutchmor, 1988; Joanisse & Storey, 1996; Bennett et al., 1997; Khani et al., 2007; Cakmak et al., 2008). Phosphatidylcholine of diapause eggs contained more linolenic acid (27% *vs.* 16%) than that of non-diapause eggs. In the overwintering larvae of the fly *E. solidaginis*, the unsaturated fatty acid/saturated fatty acids ratio (UFA/SFA) increasesin total phospholipids from 3.0 to 4.2 during autumn (Bennett et al., 1997). In contrast to most other insects, the proportion of UFAs do not increase in diapausing stage of two heteropterans, *Pyrrhocoris apterus* (Linnaeus, 1758) (Heteroptera: Pyrrhocoroidae) (Hodková et al., 1999) and *Eurygaster integriceps* (Puton, 1881) (Heteroptera: Pentatomidae) (Bashan et al., 2002).

These changes in FA percentage indicate that insect are able to modify their FA compositions, probably to suit local physiological requirements in seasonal changes. This suggests a possible regulation of the corresponding enyzme systems for the metabolism of its FA requirements. The FA

compositions can be modified by hydrolysis of some FAs, coupled with selective reacylation of others, and also by altering existing components (Stanley-Samuelson et al., 1992). The ability to elongate and desaturate PUFAs is one of the mechanisms of changing FA profiles; and such metabolic abilities are linked to physiological needs by providing C20 PUFAs. These changes may be important during the winter, because the insects does not continuously readjust its membrane FAs to maintain fluidity.

## Özet


**_Eysarcoris inconspicuous_ (Herrich-Schaffer, 1844) (Heteroptera: Pentatomidae) erginlerinin yağ asidi kompozisyonundaki mevsimsel değişimler**

Bu çalışmanın amacı, *Eysarcoris inconspicuous* (Herrich-Schaffer, 1844) (Heteroptera: Pentatomidae) erginlerinin fosfolipit ve triaçilgliseroldeki yağ asidi kompozisyonlarda mevsimsel farklılıklar sonucu meydana gelen değişimi araştırmaktır. *E. inconspicuous* erginleri Diyarbakır'dan (Türkiye) 2007-2008 yılları arasında toplanmıştır. Fosfolipit ve triaçilgliserol fraksiyonlarındaki yağ asidi kompozisyonları *E. inconspicuous*'un tüm vücudu kullananılarak izole edilmiş ve gaz kromatografisi ve gaz kromatografi-kütle spektrometresi ile analizlenmiştir. Ayların çoğunluğunda kalitatif olarak 15 yağ asidinin varlığı ortaya çıkarılmıştır. Majör olan yağ asitleri çoğu hayvanlarda da gözlenen 16 ve 18 karbonlu doymuş ve doymamış bileşenlerdir. Bu bileşenlere ek olarak, üç adet tek zincirli (C13:0), (C15:0), (C17:0) ve prostaglandinlerin öncül maddesi olan yağ asitleri de bulunmuştur. Fosfolipit ve triaçilgliserol yağ asidi profillerinde bazı farklılıklar gözlenmiştir. Triaçilgliserolün aksine fosfolipitte linolenik asit ve 20 karbonlu aşırı doymamış yağ asitlerinde sonbahar ve kış süresince artış gözlenmiştir. Her iki fraksiyonda da aşırı doymamış yağ asitlerinin oranı doymuş yağ asitlerine göre önemli derecede artış göstermesine rağmen bu artış sonbaharda ve özelikle mevsim sıcaklığının düşük olduğu ocak ve şubat aylarında fosfolipitte kendisini bariz olarak hissetirmiştir. Bu nedenle *E. inconspicuous*'un yağ metabolizmasında mevsimsel farklılıktaki sıcaklık değişiminin önemli bir rol oynadığı görülmektedir. Böcekler için düşük sıcaklıktan dolayı meydana gelen hücresel hasarı önlemek önemli bir sorundur. Bu bulgularımız, *E. inconspicuous*'un çevresel şartların değişiminden dolayı kendi yağ asidi kompozisyonunu modifiye edebildiğini göstermektedir.


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