

Comparison of Energy Reserves in Prediapause and Diapausing Adult Sunn Pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae)

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ABSTRACT

In this study, the energy reserves of prediapause and diapausing adult bugs were examined using colorimetric biochemical techniques to determine carbohydrates, lipids, glycogen, and protein content. To this end, 45-day-old bugs were obtained from three different sources: laboratory colony, cold-stored insects, or natural habitat. The results showed that prediapause males and females had significantly lower lipids than laboratory cultured bugs, those were collected in natural habitat, and cold-treated diapausing insects. In contrast to lipids, carbohydrate and glycogen contents were significantly higher in prediapause males and females than in diapausing insects. Glycogen content in laboratory-reared females and males were significantly higher than in their counterparts from natural habitat. In conclusion, Sunn pest energy reserves change from prediapause to diapause phases. Prediapause Sunn pest bugs accumulate lipids for their metabolic needs during diapause and for post-diapause functions that include dispersal and reproduction.

Keywords: Carbohydrate, Glycogen, Lipid, Protein, Sunn pest.

INTRODUCTION

Sunn pest (*Eurygaster integriceps* Puton) (Hemiptera: Scutelleridae), is a serious pest of cereals in the Near and Middle East, eastern and southern Europe, and North Africa (Radjabi, 2000). This insect causes severe quantitative and qualitative damage to cereals by feeding on leaves, stems, and grains, sometimes causing up to 100% yield loss.

The Sunn pest has one generation per year, thus, it has a univoltine life cycle which has an obligatory reproductive diapause in each generation regardless of environmental conditions. This diapause involves arrested development of female ovaries and inhibition of egg maturation and deposition. Males undergo a weak diapause, which involves delayed maturity of testes and sperm production until almost 45 days post-

emergence. Shinyaeva (1980) studied spermatogenesis in young males of the Sunn pest during prediapause and onset of diapause and it was found not to be completed by the time of wing formation, but at some later stage of development. The onset of diapause did not arrest spermatogenesis; bundles of late spermatids and spermatozoa were formed and reoriented spatially during diapause. The testes of bugs terminating hibernation contained mature sperms.

The life cycle of this insect has two distinct phases: growth and development take place on wheat, whereas diapause occurs in a different habitat, such as oak forest litter in Europe, or in bushes of *Artemisia* spp. or *Astragalus* spp. in the mountains of Asia (Paulian and Popov, 1980). The long diapause period consists of two distinct phases, aestivation and hibernation (Paulian and Popov, 1980;

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Radjab, 2000). Thus, the species is in diapause for about eight months and is active only for about four months in spring and early summer.

Diapause is a hormonally controlled cessation of development that is a metabolically expensive life history strategy (Hahn and Denlinger, 2007, 2011; Emerson *et al.*, 2009). Among insects that diapause as adults, nutrient reserves are critical for restoring post-diapause functions including dispersal and reproduction (de Kort, 1990). Insects have to expend energy constantly, and if they are not feeding, they must live on reserves that have been accumulated in prior periods of food abundance. Energy reserves may be stored in animal cells as glycogen or triglycerides. Glycogen is a polymeric form of glucose that can be depolymerized on demand to be used as fuel (Steele, 1985). Lipids are the most important reserves used by insects to meet their energy demand during diapause (Hahn and Denlinger, 2007) and to fuel prolonged periods of flight (Beenackers *et al.*, 1984) as they contain almost four times the energy per unit mass as sugars. Newly emerged adults of Sunn pest (prediapause adults) feed on wheat and store lipids in spring and then use these to support their energy demands during estivation and hibernation (Bashan *et al.*, 2002). Since there is no information regarding energy reserve of Sunn pest during prediapauses and diapausing periods, this research was carried out to compare energy reserves among laboratory-reared prediapause bugs (newly emerged adults that becomes destined for later entry into the diapauses phase) (3-day-old adults), diapausing bugs (45-day-old adults), bugs held under cold conditions (4°C), and adults collected from natural habitat.

MATERIALS AND METHODS

Insect Colony

Eurygaster integriceps adults were collected from a wheat field in Karaj, Alborz Province, Iran, in mid spring. The stock

colony of *E. integriceps* was maintained in the laboratory under 16L:8D photoperiod at 26±1°C and 55±5% RH on soaked wheat kernels as described by Bandani *et al.* (2009) and Allahyari *et al.* (2010).

Experimental Insects

Laboratory reared bugs were tested as three-day-old adults (= Prediapause adults) and as 45-day-old adults (= Diapausing adults). The adult insects were fed during the 45-day period after final molting. Also, some adults (300 individuals, ≈45-day-old) were collected from natural habitat (under bushes of *Artemisia* or *Astragalus* spp. in the mountains of Karaj) where they were in diapause. For each group of insects, three replicates of 10 insects (five females and five males) were tested.

Another group of bugs (< 24 hour-post molt adults) were collected from stock colonies, placed in plastic boxes, and held in a cold room set at 4°C for 45 days. To serve as the control insects, another group of bugs (< 24 hour-post molt adults) were maintained at 26°C for 45 days before testing. Feeding was arrested in bugs receiving cold treatment, but laboratory cultured insects continued feeding during this period.

Protein Determination

Prior to analysis, each individual was weighed and homogenized in 400 µl of distilled water, centrifuged at 8,000g for 10 minutes at 4°C, then supernatant was taken for protein determination. Protein content was quantified as described by Bradford (1976) using bovine serum albumin (BSA) as a standard.

Lipid Determination

Lipid determination was done following Yuval *et al.* (1998). Briefly, each Sunn pest was homogenized in 400 µl of 2% Na₂SO₄. Then, 1,300 µl of chloroform: methanol (1:2)

was added to the homogenate before centrifugation at 8,000g for 10 minutes at 4°C. For lipid determination, 50 µl was taken from each supernatant and dried in a water bath at 90°C. Samples were then dissolved in equal amounts (50 µl) of concentrated H₂SO₄ and heated for 10 minutes at 90°C. Subsequently, 15 µl was added to 135 µl of vanillin reagent (600 mg vanillin dissolved in 100 ml distilled water and 400 ml 85% H₃PO₄). The plate was incubated for 30 minutes at room temperature and then the optical density was read at 530 nm on an ELx 808™ Bio-tek Spectrophotometer. Total content of lipids in each individual was calculated from a standard curve of cholesterol.

Carbohydrate Determination

Carbohydrate was determined according to Yuval *et al.* (1998). Briefly, each Sunn pest was homogenized in 400 µl of 2% Na₂SO₄. Then, 1,300 µl of chloroform: methanol (1:2) was added to the homogenate before centrifugation at 8,000g for 10 minutes at 4°C. Thirty µl was taken from the supernatant and 20 µl of water was added to it. Then, 100 µl anthrone reagent (500 mg anthrone dissolved in 500 ml concentrated H₂SO₄) was added to the mixture and heated 15 minutes at 100°C in water bath and the optical density was read at 630 nm. Total content of carbohydrates in each individual was calculated from a standard curve of glucose.

Glycogen Determination

Glycogen measurement was also done according to the method of Yuval *et al.* (1998). Briefly, each Sunn pest was homogenized in 400 µl of 2% Na₂SO₄. Then, 1300 µl of chloroform: methanol (1:2) was added to the homogenate before centrifugation at 8,000g for 10 minutes at 4°C. The pellet was washed in 400 µl 80% methanol, thus removing possible remnants of sugar. To extract the glycogen, 250 µl of water was added to the washed pellet and heated for 5 minutes at

70°C. Then, 125 µl anthrone reagent (600 mg anthrone dissolved in 300 ml concentrated H₂SO₄) was added to 25 µl of the supernatant and the solution was incubated at 90°C for 10 minutes. Optical density was read at 630 nm and total glycogen in each individual was calculated from a standard curve of glycogen.

Statistical Analysis

Data were subjected to one-way and two-way analyses of variance (ANOVA), followed by LSD test to separate means when differences were significant at $P \leq 0.05$.

RESULTS

Prediapause versus Diapausing Adults

Most energy reserves in prediapause bugs were in the form of carbohydrates (84.97% of total); however, diapausing adults had more lipids (57.71% of total energy

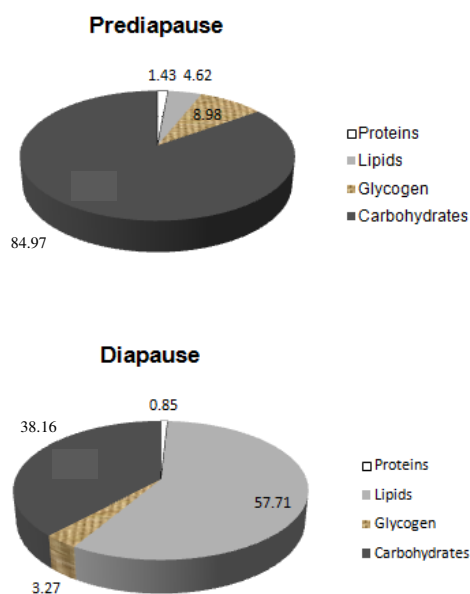


Figure 1. Comparison of energy reserves (%) contents of prediapause (3-day-post molt adult) and diapausing (45-day-post molt adult) of *Eurygaster integriceps*.



reserves) (Figure 1). Prediapause males and females (three-day-old adults) had significantly lower lipids than laboratory (45-day-old adult) diapausing insects ($P < 0.05$) (Figure 2). For example, lipid content of prediapause male and female was 1.19 and 0.35 mg g^{-1} of fresh adult mass, respectively. However, lipid content of diapausing male and female was 18.06 and 11.90 mg g^{-1} , respectively (Figures 2-A and 2-B). Lipid content in males was higher than in female adults e.g., in prediapause state, females' lipid content was 29.48% of that of males; while in diapausing state, females' lipid content was 65.89% of that of males.

Total carbohydrate and glycogen contents were significantly higher in prediapause males and females than diapausing insects ($P < 0.05$) (Figures 2-A and 2-B). However, there was no significant difference in carbohydrate and glycogen contents between sexes. Total carbohydrate in male and female prediapause adults (3-day-old) was 15.18 and 11.43 mg g^{-1} , respectively, while in male and female diapausing adults (45-day-old), it was 9.01 and 10.32 mg g^{-1} ,

respectively. Also, glycogen content in male and female prediapause adult (3-day-old) was 1.59 and 1.22 mg g^{-1} , respectively, while in male and female diapausing adult (45-day-old), it was 0.69 and 0.95 mg g^{-1} , respectively (Figures 2-A and 2-B). There was no significant difference in protein content between prediapause and diapausing insects, nor between sexes (Figures 2-A and 2-B).

Cold Treated versus Wild-collected Adults

Cold treated insects showed high mortality (up to 90%). Thus, further experiments were carried out on the live individuals as follow. Total energy reserves (lipids+carbohydrates+glycogen+proteins) of cold exposed Sunn pests were significantly lower than laboratory reared control (65.55% of the control's total energy reserves) and natural habitat collected adults. However, there were no significant differences in energy reserve of laboratory cultured insects (control) and natural habitat collected adults (95.52% of control's total energy reserves).

Cold exposed Sunn pests had higher lipids/total energy reserves ratio (78.8%) than the control (laboratory reared Sunn pests) (57.71%) and natural habitat collected adults (68.91%). In all treatments, proteins were the lowest energy reserves, i.e. 0.85, 1.42, and 0.94% of total energy reserves of laboratory reared-, cold treated- and natural habitat collected insects, respectively (Figure 3).

Due to decrease in total energy reserves of cold exposed Sunn pests, within each sex, there was no significant difference in lipid contents between different treatments, i.e. 45-day-old adults of laboratory reared-, cold treated- and natural habitat collected insects (Figure 4-A). Male's lipid contents in laboratory reared-, cold treated-, and natural habitat collected insects were 18.06, 19.58, and 18.75 mg g^{-1} , respectively. In the case of females, lipid contents in laboratory reared-, cold treated-, and natural habitat collected

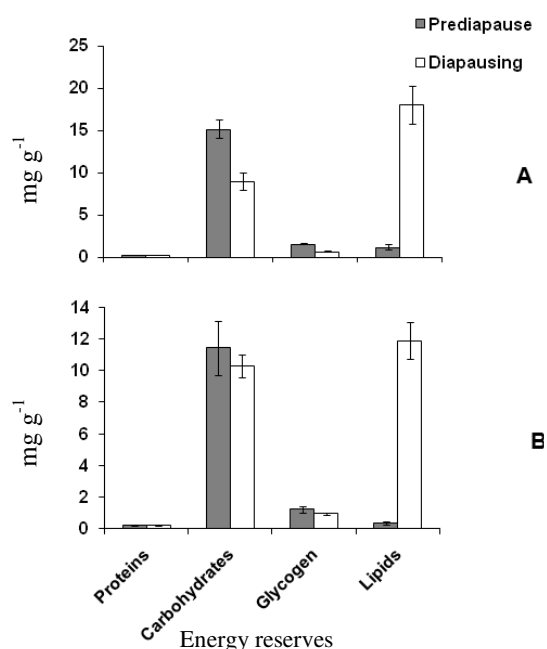


Figure 2. Energy reserves content (mg g^{-1} fresh mass) of prediapause (3-day-post molt adult) and diapausing (45-day-old) adult males (A) and females (B) of *Eurygaster integriceps*.

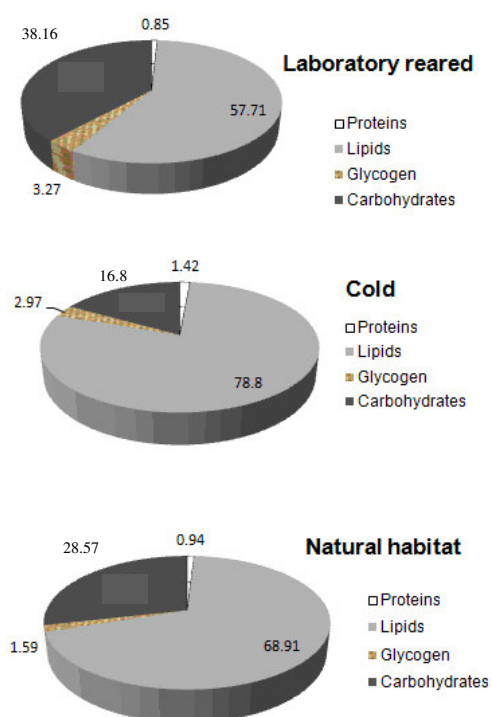


Figure 3. Comparison of energy reserves (%) contents of diapausing laboratory reared, cold exposed-, and natural habitat collected adults (45-day-post molt) of *Eurygaster integriceps*.

insects were 11.90, 6.96, and 15.07 mg g⁻¹, respectively. However, in all treatments in which females' lipid content was lower than that of males, the difference was significant ($P < 0.01$) (Figure 4-A). For example, lipid content in cold treated males was 19.58 mg g⁻¹, whereas for the corresponding females it was 6.96 mg g⁻¹.

There was no significant difference in carbohydrate, glycogen, and protein contents between sexes in all treatments.

Carbohydrate content among cold exposed insects and the other treatments was significantly different ($P < 0.05$) (Figure 4-B). Cold exposed adults had significantly lower carbohydrates than laboratory cultured and natural habitat insects (Figure 4-B). Female's carbohydrate content in cold exposed-, laboratory reared-, and natural habitat insects was 2.61, 10.32, and 6.53 mg g⁻¹, respectively, while in males it was 3.05, 9.01, and 7.49 mg g⁻¹, respectively.

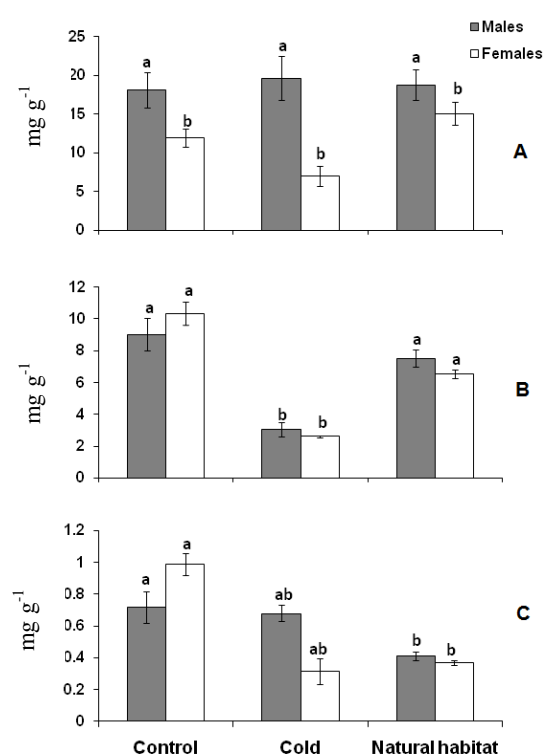


Figure 4. Total lipid (A), total carbohydrate (B), and glycogen (C) contents (mg g⁻¹ fresh mass) of diapausing laboratory cultured, cold exposed and natural habitat collected adults of *Eurygaster integriceps*.

45-day-old adults as diapausing insects were chosen. Lipid content was significantly lower in females than males ($P < 0.01$). Means followed by different letters are significantly different at $P < 0.05$ using LSD tests.

Glycogen content in laboratory reared insects was significantly higher than natural habitat insects ($P < 0.05$) (Figure 4-C). Glycogen contents in laboratory reared females and males (0.99 and 0.72 mg g⁻¹, respectively) were significantly higher than natural habitat counterparts (0.37 and 0.41 mg g⁻¹, respectively).

Cold exposed males and females had lower glycogen (0.68 and 0.32 mg g⁻¹, respectively) than laboratory reared insects (0.72 and 0.99 mg g⁻¹, respectively), but the difference was not statistically significant. In all treatments, less than 50% of carbohydrate

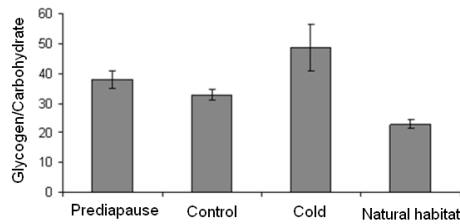


Figure 5. Glycogen/Carbohydrates ratio in prediapause adults and diapausing laboratory cultured, cold exposed, and natural habitat collected adults of *Eurygaster integriceps*. Three-day-old adults, as prediapause insects, and 45-day-old adults, as diapausing insects, were chosen. In all treatments, less than 50% of carbohydrate content was glycogen.

content was glycogen (Figure 5). The highest glycogen/carbohydrate ratio was seen in cold exposed insects and the lowest glycogen/carbohydrate ratio was observed in natural habitat insects (Figure 5). Glycogen/carbohydrate ratio in prediapause insect was almost the same as laboratory reared (control) and the cold exposed insects.

There was no significant difference in protein content among different treatments i.e. cold exposed-, laboratory reared-, and natural habitat adults (Figure 6).

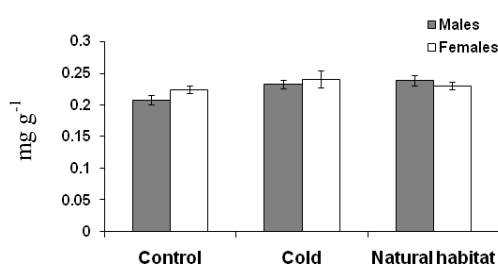


Figure 6. Protein contents (mg g^{-1} fresh mass) of diapausing laboratory cultured, cold exposed, and natural habitat collected adults of *Eurygaster integriceps*. 45-day-old adults, as diapausing insects, were chosen. There was no significant difference neither in protein content among treatments nor between sexes.

Energy Comparison

As shown in Table 1, based on energy conversion indices of Withers (1992), male individuals had more available energy than females. In the case of males, available energy of prediapause, laboratory reared, cold treated, and natural habitat collected insects were 330, 851, 807, and 852 Joule g^{-1} , respectively, while in females, available

energy was 228, 643, 314, and 724 Joule g^{-1} , respectively. Prediapause male and female adults had less available energy than diapausing male and female adults in all treatments including laboratory reared, cold treated, and natural habitat.

DISCUSSION

For the first time, this study shows how the form of energy reserves changes over different phases of adult life in *E. integriceps*. As diapause proceeds, the amount of carbohydrate and glycogen decreases but lipid content increases (Critchley, 1998). For example, lipid content of prediapause males and females was 1.19 and 0.35 mg g^{-1} of fresh mass, respectively. However, lipid content of diapausing males and females was 18.06 and 11.90 mg g^{-1} , respectively. Also, the amount of carbohydrate and glycogen with development of diapauses decreased. These data show that in diapausing Sunn pest carbohydrates and glycogen convert to lipids. It has been reported that carbohydrates, a major component of diet in insects including Sunn pest, are converted into lipids in their fat body (Hines and Smith, 1963; Bailey, 1975; Venkatesh and Morrison, 1980; Inagaki and Yamashita, 1986; Briegel, 1990). Insect fat body has high capacity for the synthesis of lipids than glycogen and this may explain the higher content of lipids compared to glycogen in the insect fat body. For example, it was reported that in female mosquito *Aedes aegypti* (L.), about 50 and 35% of the diet glucose was used for

Table 1. Energy Comparison of reserves mass of males and females individual in different treatments.

Energy (J g ⁻¹)	Male					Female				
	Lipid	Carbohydrate	Glycogen	Protein	Available energy	Lipid	Carbohydrate	Glycogen	Protein	Available energy
Prediapause	45.22	258.06	27.03	5.06	330.31	13.3	194.31	20.74	4.83	228.35
Laboratory reared	686.28	153.17	11.73	4.83	851.18	452.2	175.44	16.15	5.29	643.79
Cold treated	744.04	51.85	11.56	5.29	807.45	264.48	44.37	5.44	5.52	314.29
Natural habitat	712.5	127.33	12.24	5.52	852.07	596.6	111.01	16.83	5.29	724.44

The indices used for energy conversion for carbohydrate, lipid, protein, and glycogen were 17, 38, 23, and 17, respectively, based on Withers (1992).

synthesis of lipids and glycogen, respectively (Zhou *et al.*, 2004). Lipid metabolism

produces more metabolic water and energy than any other molecule.

Interestingly, even in cold treated adult insects that had been placed at 4°C at the age of less than 24 hours, after 45 days, lipid content increased while carbohydrates and glycogen decreased. It seems that the insects accumulate lipids to protect themselves from cold adverse effects. However, high mortality (up to 90%) in cold treated adults suggests that Sunn pest accumulate lipid because of predetermined genetic program to spend a long period of overwintering diapause and prepare itself to produce a new generation.

Since the adult Sunn pest has a long flight from overwintering site to wheat fields, conversion of carbohydrates to lipids makes sense in term of flight energy demand. Also, insects that accumulate more lipids in their epicuticle have more capacity to withstand desiccation (Danks, 2000), and those that have more lipids have more fuels available for post-diapause adult activity, thus enhancing reproductive fitness in the following season (Ellers and van Alphen, 2002). It has been reported that diapausing individuals of some insect species accumulate more fat or glycogen reserves than non-diapause individuals as part of the diapause preparatory program, e.g. last instar diapause larvae of *Pectinophora gossypiella* (Saunders) accumulate 50% more lipid reserves than non-diapausing last instar larvae and diapause-destined adult *Culex pipiens* (L.) accumulate almost twice the lipid and carbohydrate stores as equivalently aged non-diapause adults (Adkisson *et al.*, 1963; Mitchell and Briegel, 1989; Bowen, 1992).

Glycogen content reduction may be due to direct utilization of this compound for energy generation either for cold-induced innutrition or for flight. Glycogen is rapidly catabolized, resulting in a rapid decrease in this energy reserve (Rambabu and Rao, 1994; Sancho *et al.*, 1998). Production of trehalose and sugar alcohols (polyols) is also induced from glycogen under stressing conditions of



temperature which are needed for adaptation to cold (Storey, 1997; Worland *et al.*, 1998).

Adult insects collected from natural habitat had significantly lower glycogen than laboratory cultured insects. It seems likely that they expended some glycogen reserves during migratory flight to overwintering sites.

Protein content of prediapause and diapausing insects including cold exposed-, laboratory reared-, and natural habitat adults was not different, indicating that this insect species may not use it as an energy reserve. It has been reported that some insects such as *Glossina* spp. (Dip: Glossinidae) and *Leptinotarsa decemlineata* (Col: Chrysomelidae) use protein (proline) as an energy source (Niaqi *et al.*, 1992).

Available energy of both male and female prediapause adults was lower than diapausing adults in all treatments, reflecting changes in metabolic activity of the insects during diapause period. Although cold treated insects did not feed during storage, they had much more available energy than prediapause adults, indicating that these individuals convert other energy reserves to lipid thus making it more suitable for them to have more available energy (Bashan *et al.*, 2002).

In conclusion, it can be said that Sunn pest energy reserve changes dramatically during diapause period. These insects use carbohydrate content, especially glycogen, to supply energy for migratory flight to overwintering sites. Besides, as diapause proceed even when adult insects are placed at 4°C, carbohydrate and glycogen content of the insect body decrease and, instead, the amount of lipid content of the body increases. Lipid accumulation plays vital role in successful diapause termination; therefore, interference in the insect lipogenesis can be used in pest control programs.

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***Eurygaster integriceps* Puton** مقایسه ذخایر انرژی در حشرات کامل سن گندم
(Hemiptera: Scutelleridae) پیش دیاپوز و در حال دیاپوز

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چکیده

در این مطالعه، ذخایر انرژی حشرات کامل پیش دیاپوز و در حال دیاپوز با استفاده از تکنیکهای کالری متریک بیوشیمیایی مورد بررسی قرار گرفتند تا مقدار کربوهیدرات، لیپید، گلیکوژن و پروتئین تعیین شود. سن ها ۴۵ روزه بودند و از سه منبع مختلف شامل حشرات پرورش یافته در آزمایشگاه، حشرات نگهداری شده در سرما، یا حشرات جمع آوری شده از زیستگاه طبیعی بودند. نتایج نشان داد که حشرات نر و ماده پیش دیاپوز، نسبت به حشرات در حال دیاپوز پرورش داده شده در آزمایشگاه، جمع آوری شده از زیستگاه طبیعی و در معرض سرما قرار گرفته، به طور معنی داری لیپید کمتری داشتند. در مقایسه با لیپید، مقدار کربوهیدرات و گلیکوژن در نرها و ماده های پیش دیاپوز، به طور معنی داری بیشتر از حشرات در حال دیاپوز بود. مقدار گلیکوژن در ماده ها و نرهای پرورش داده شده در آزمایشگاه، به طور معنی داری بیشتر از آنهایی بود که در زیستگاه طبیعی بودند. به طور کلی میتوان گفت که ذخایر انرژی سن گندم از مرحله پیش دیاپوز تا دیاپوز، به طور چشمگیری تغییر میکند. سن گندم پیش دیاپوز، چربی را برای نیازهای متابولیک خود در عملکردهای دوران دیاپوز و پس از دیاپوز شامل پراکنش و تولید مثل انباشته میکند.