EFFECT OF DIET, PHOTOPERIOD AND COLD ON *EURYGASTER INTEGRICEPS* PUTON DIAPAUSE (HEMIPTERA: SCUTELLERIDAE)

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ABSTRACT: Sunn pest (*Eurygaster integriceps* Put.) (Hemiptera: Scutelleridae), is a serious pest of cereals causing severe quantitative and qualitative by feeding on leaves, stems and grains. Aim of the current study was to evaluate the effect of photoperiod and diet on the Sunn pest diapause. Thus, the effect of two photoperiod conditions including short day (8 L: 16D) and long day (16L: 8D) and two diet including wheat grains and growing wheat plants on stadium duration, gonad size and haemolymph protein concentration was examined. Also, to extend our studies the effect of cold on gonad size and haemolymph protein concentration was studied. Results showed that stadium duration in short day photoperiod condition (31.43 days) was shorter than long day photoperiod condition (38.58 days) ($P<0.05$) i.e. when the nymphs were placed in short day conditions and fed on wheat grains their development time (time taken to adult) was shortened about 15–16 days in compare with those nymphs that placed in long day conditions and fed on wheat plants. Short day photoperiod condition and wheat grain diet caused larger ovary size (4.08 ± 0.48 mm^2) than long day conditions and wheat plant diet (3.12 ± 0.04 mm^2). Cold exposed females and males had lower haemolymph protein concentration than control females and males. The results showed that although photoperiod and diet cause significant changes on the stadium duration, ovaries and testis size and haemolymph electrophoretic bands, their distinct effects on diapause needs to be elaborated more.

KEY WORDS: Sunn pest, Diapause, Diet, photoperiod, cold.

Sunn pest (*Eurygaster integriceps* Put.) (Hemiptera: Scutelleridae), is a serious pest of cereals in the wide area of the globe from Near and Middle East to East and South Europe and North Africa (Radjabi, 2000). The insect causes severe quantitative and qualitative (destruction of gluten protein) damage to crops (sometime up to 100%) by feeding on leaves, stems and grains.

E. integriceps has a monovoltine life cycle, with diapause occurring at the adult stage. The life cycle develops in two different phases in which growth and development take place in wheat, whereas diapause (aestivation and hibernation) 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 & Popov, 1980). The long diapause period is divided into two distinct steps, aestivation and hibernation (Paulian & Popov, 1980; Radjabi, 2000). So it can be said that the insect is about nine months in diapausing state and it is active only in spring for about three months.

Diapause is an adaptive mechanism for dormancy during periods of unfavorable environmental conditions (Tauber et al., 1986). Diapause is defined as physiologically controlled suppression of growth, development or reproduction. Diapause and non-diapause individuals differ in developmental, physiological, morphological and behavioral characteristics (Tauber et al., 1986; Danks, 1987). One of the most important features of adult diapause is a cessation of reproduction. Thus, when insects have adult diapauses, vitellogenesis does not take place after emergence (Danks, 1987). As a result immature ovaries have been used as the criterion of adult diapauses and in males which experience the diapause the testes are reduced in size, but in other cases the testes remain well developed (Denlinger, 2000; Numata & Hidaka, 1983).

Many insects use environmental cues such as photoperiod or temperature and respond with specific physiological, behavioural and morphological modifications that enable them to survive adverse conditions in a state of reduced metabolism (Saunders, 1982; Tauber et al., 1986). Photoperiod plays a major role in diapause induction in many species of insects, particularly those from temperate regions (Beck, 1980; Saunders, 1982).

Quality and quantity of food affect incidence of diapauses in insects (Tauber et al., 1986; Danks, 1987; Overmeer et al., 1989; Fielding, 1990). A restricted intake of nutrients can indirectly lead to the induction of diapause due to a delay in development (Clay & Venard, 1972; Saunders & Bradley, 1984).

Some coleopteran insects such as Colorado potato beetle (*Leptinotars decemlineata*) and hemipteran insects such as Sunn pest (*E. integriceps*) enter diapause in the adult stage. In these insects, ovarian development is arrested in diapausing females, and the diapause is called a reproductive diapause (Okuda & Chinzei, 1988).

Since little is known about factors affecting diapause in Sunn pest this research was undertaken to study the effect of two different diet including wheat kernels (grains) and growing wheat plants in two different photoperiods including long day photoperiod (16L: 8D) and short day photoperiod (8L: 16D) on the induction of adult Sunn pest reproductive diapause.

In the current study differences in reproductive organ size and haemolymph protein concentration under long- and short day photoperiods and two different diets were used in the determination of diapause induction.

Also, developmental duration of *E. integriceps* from egg to adult emergence and the fertility of the females (egg laying activity) were determined under long- and short day photoperiods and two different diets.

The gained knowledge is essential for understanding the seasonal biology of the Sunn pest, and such information is required for the development of effective pest management strategies of the species.

MATERIALS AND METHODS

Maintenance of insects' colony

E. integriceps adults have been collected from the Karaj wheat field, Tehran Province, Iran. 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).

Photoperiod and diet treatment

To determine the effect of photoperiod and diet on diapause, eggs (5 day old) were placed under either long-day photoperiod (16L: 8D) or short day photoperiod (8L: 16D) each in two diet regimens including wheat grains and growing wheat plants prior to their ear-bearing stage at 26±1°C and 55% RH. Each treatment was repeated three times each replication with 80 eggs. The growing plants were replaced when wilted or yellowed.

The following parameters including development time to the adulthood, gonad size using drawing tube, haemolymph protein concentration were examined in adult females and males at 1 day and 7day post emergence.

Cold treatment

To test the effect of cold treatment on diapause, adult females and males (<24-h-old) were collected from the stock colony and were transferred to 4°C one day post-emergence. During the cold experiment, the insects did not receive food and water.

Also some diapausing adults (≈45-day-old) collected from the natural habitat where they were in diapausing state and their gonad size was determined.

Control insects (<24-h-old) were collected from the stock colony and were maintained at normal rearing conditions (in laboratory under 16L: 8D photoperiod at 26 ±1°C and 55 ± 5% RH).

Adults (controls and cold exposed adults) were dissected in distilled water at 15, 30 and 45 days post-exposure and gonad size was measured. Individual adult insect after cold-exposed time (15, 30, 45 days) was transferred to normal rearing condition (26 ±1°C) in order to feed and then after three days their haemolymph protein was measured. Transferring to normal rearing condition was done since taking blood from starved insect was problematic.

Determination of haemolymph protein concentration

Protein concentration was measured according to the method of Bradford (1976), using bovine serum albumin (Bio-Rad, Munchen, Germany) as a standard. For protein determination, haemolymph from the control and treated adults (females and males) was collected in a chilled calibrated micro capillary pipette through amputated forelegs and diluted (1:1) with anticoagulant buffer (41 mM citric acid, 1.7 mM EDTA, 98 mM NaoH and 186 mM Nacl; pH 4.5). The samples were centrifuged at 10000g for 10 min at 4°C to remove haemocytes and other tissue fragments. The resulting supernatants were stored at -20°C for further analyses.

Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on 10% slab gels according to the Laemmli (1970). Samples were diluted (1:1) in sample buffer (0.5 M Tris–HCl, pH 6.8, 10% SDS, Glycerol, 2-mercaptoethanol), boiled for 5 min and loaded into the gel along with bromophenol blue as tracking dye. Gels were run in Tris–glycine buffer (Tris base, SDS, glycine, pH 8.3). Following electrophoresis, gels were stained in 0.1% coomassie brilliant blue R-250 in 40% methanol and 10% acetic acid at room temperature. Gels were then destained in 40% methanol and 10% acetic acid until bands appeared.

Statistical Analysis

Data were compared by one-way and two-way analysis of variance (ANOVA), followed by LSD multiple range test when significant differences were found at $P \leq 0.05$. Software SAS was used for all statistical analyses.

RESULTS

Effect of photoperiod and diet treatment on stadium duration

Photoperiod condition caused significant difference in development time of nymphs when reared on both wheat grains and growing wheat plant ($P<0.05$).

Stadium duration in short day photoperiod condition (31.43 days) was shorter than long day photoperiod condition (38.58 days) ($P<0.05$) i.e. When the nymphs were placed in short day conditions (8 L: 16D) and fed on wheat grains their development time (time taken to adult) was shortened about 15–16 days in compare with those nymphs that placed in long day conditions and fed on wheat plants.

Short day photoperiod condition and wheat grain diet caused a significant decrease in the developmental duration of *E. integriceps* (28.59 days) compared to the other regimes ($P<0.05$). Development time to adulthood in short day photoperiod condition and wheat plant diet with long day photoperiod condition and wheat grain diet was almost similar (≈ 34 days). Long day photoperiod condition and wheat plant diet caused longer developmental duration (43.04 days) (Fig 1).

Effect of photoperiod and diet treatment on gonad size

As early as the first day of imaginal life, the size of the reproductive organ was different in examined regimes (Table 1). In both sexes, adults promote development of their reproductive organs under wheat grain diet. However, there was not significant difference in ovarian length among regimes. The mean width of ovary in long day condition and wheat grain diet was higher than other regimes ($P<0.05$). Short day condition and wheat grain diet caused larger ovary size (Size = Width X Length) than long day conditions and wheat plant diet ($P<0.05$). The mean width, length and size of testis in growing wheat plant reared Sunn pest was smaller than wheat grain reared insects ($P<0.05$) (Table 1).

Effect of photoperiod and diet treatment on haemolymph protein concentration

There was not significant difference in haemolymph protein concentration between genders (Fig. 2). For example, protein concentration in insects reared on short day- and long day photoperiod on wheat grains on first day was 0.12 and 0.14 mg ml^{-1}, respectively. However, total protein concentrations increased over time ($P<0.01$). Protein concentration in long day photoperiod on wheat grains at first and seven days was 0.12 and 0.25 mg ml^{-1}, respectively.

Effect of cold treatment on gonad size

There was not significant difference in ovarian width and length among insects in different days in different treatments i.e. 15-, 30- and 45 days cold exposed adult. However, ovarian length was increased in control insects (at normal rearing conditions (in laboratory under 16L: 8D photoperiod at 26 ±1°C and 55 ± 5% RH) at 45 days after adult emergence ($P<0.05$) (Table 2). Cold exposed females had larger ovary than control ($P<0.05$). Ovary size in females collected from natural habitat was similar to control insects (laboratory reared insects).

Testis size increased in control males over time, but its development did not occur in cold exposed males ($P<0.01$). Also there was not significant difference in gonad size between laboratory reared and insects collected from natural habitat (Table 2).

Effect of cold treatment on haemolymph protein concentration

Total protein concentration declined in cold treated insects. Cold exposed females and males had lower haemolymph protein concentration (0.185±0.01 and 0.197±0.005 mg/ml respectively) than control females and males (0.293±0.007 and 0.277±0.048 mg/ml respectively) ($P<0.01$). There was not significant difference in haemolymph protein concentration between genders in control. However, cold exposed females had lower haemolymph protein than males

(P<0.01) (Fig.3). Some protein bands of control haemolymph did not observe in cold exposed insects haemolymph (Fig. 4).

DISCUSSION

Photoperiod is a reliable signal for insects to indicate coming winter season. So, insects of the temperate zones often use photoperiod to predict unfavorable conditions. In the current study the effect of photoperiod conditions such as long and short day on the insect stadium duration and future adult reproductive diapause was assessed. Results showed that short day photoperiod condition reduced nymphal duration by almost 7 days indicating that insect use the short day signals to predict unfavorable condition as a result they speed up their growth. However, it did not significantly affect the insect reproductive diapause.

Short day photoperiod induced reproductive diapause in *Scotinophara lurida* (Hemiptera: Pentatomidae) (Cho et al., 2008) and another pentatomid, *Graphosoma lineatum* (Nakamura et al., 1996). Solbreck (1979) showed that when laboratory cultures of third or fourth instar nymphs of *Neacoryphus bicrucis* (Say) (Lygaeidae) were moved from a 16L: 8D to 12L: 12D photoperiod condition, diapause occurred in 66% of adult females reared from third instar nymphs and in 43% of adult females reared from fourth instar nymphs.

Some insects that have summer diapause they use long day lengths instead of short day lengths for entering diapause (Masaki, 1980). The brown stink bug, *Euschistus heros* showed better reproductive performance at the long day photoperiod and enters reproductive diapause at short day photoperiod (Mourao & Panizzi, 2002). *Pyrrhocoris apterus* shows ovarian diapause in short day photoperiod; whilst normal reproduction occurs in long day photoperiod (Hodkova, 1975).

Food has an influence on many aspects of diapause (Tauber et al., 1986). Food presence (Fielding, 1990) and food quality (Overmeer et al., 1989) are the factors that contribute to the diapause decision. In the current study it was found that food type (wheat grain and growing wheat plant) did not affect diapause induction significantly but it affected stadium duration (lower nymphal development time).

Interestingly, ovary and testis size was affected by photoperiod and diet. Results showed that when the insects placed in short day conditions and on wheat grains their ovary size increased indicating that wheat grain had more nutrients and perhaps it prevented induction of diapause in the adult. However, it did not affect significantly the insect diapause. Female adults of *Protophormia terraenovae* produced normal ovaries under non-diapausing conditions, whereas their ovarian diapause occurred under diapause-inducing conditions (Shiga & Numata, 2001). Short day length and low temperature induced female diapause in *Oncopeltes fasciatus*. However, this condition did not induce diapause in the male (Caldwell & Dingle, 1967; Dingle, 1974).

Environmental temperature is a key factor in determination of physiological process on ectotherm species (Honek & Kocourek, 1990; Chapman, 1998). Temperature determines the biological performances of insects, such as development time of pre-imaginal stages, life expectation and reproductive parameters of the adults, induction and termination of diapause, having by this way a differential contribution of each single individual for the population growth (Kontodimas & Stathas, 2005; Cabral et al., 2006). The rate of diapause development is often influenced by temperature (Tauber et al., 1986).

It has been postulated that in a heteropteran bug, *Pyrrhocoris apterus*, termination of diapause was stimulated by cold (Kostal et al., 2008).

During cold exposure and forced starvation, total amino acid levels often increase in insects (Hanzal & Jegorov, 1991) and is believed to play a major role in cold- hardening. In our experiment protein concentration and the number of protein bands in the cold exposed females and males of the Sunn pest were less than those of controls. Thus, these insects could not resist cold treatment and died during the experiment.

In conclusion it should be said that diapause is a state of markedly decreased metabolism and activity, as well as reproductive arrest, is induced by changes in photoperiod and temperature, and is orchestrated hormonally (Denlinger, 1983). So the photoperiod changes did not affect diapause induction in the Sunn pest. Although, it has been said that cold affect Sunn pest diapause, current study showed that cold did not affect considerably diapause induction in the Sunn pest.

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Table 1. Gonad size (Mean ±se) of adult males and females in different diet and photoperiod.

Regimes	Ovary Size (mm)				Testis Size (mm)			
	Day 1		Day 7		Day 1		Day 7	
	Width	Length	Width	Length	Width	Length	Width	Length
LD-W	2.02±0.02a	1.87±0.09b	1.583±0.10b	1.73±0.04b	1.04±0.02a	1.17±0.02a	1.04±0.05a	1.14±0.09a
SD-W	1.81±0.19ab	2.25±0.14a	1.81±0.09ab	1.81±0.14b	1.15±0.05a	1.25±0.06a	1.15±0.05a	1.19±0.04a
LD-G	1.65±0.04b	1.90±0.02b	_	_	0.90±0.02b	0.92±0.02b	_	_
SD-G	1.54±0.02b	2.12±0.09ab	_	_	0.77±0.02b	0.79±0.02b	_	_

LD-W: Long day condition and wheat grain diet, SD-W: Short day condition and wheat grain diet, LD-G: Long day condition and wheat plant diet, SD-G: Short day condition and wheat plant diet. Means that followed by different letters are significantly different at P < 0.05 using LSD tests.

Table 2. Mean (±se) gonad widths and Length of cold exposed, laboratory reared and natural habitat adult males and females.

Treatments	Day 15				Day 30				Day 45			
	Ovary Size (mm)		Testis Size (mm)		Ovary Size (mm)		Testis Size (mm)		Ovary Size (mm)		Testis Size (mm)	
	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length
Control	1.46±0.07b	1.62±0.06b	1.14±0.05bc	1.21±0.08c	1.69±0.14ab	1.73±0.10b	1.42±0.10b	1.75±0.00a	1.96±0.11ab	2.17±0.10a	1.83±0.04a	1.87±0.00a
Cold-exposed	2.08±0.22ab	2.27±0.12a	0.94±0.04c	1.17±0.04c	1.71±0.09ab	2.12±0.04a	1.02±0.04c	1.17±0.02c	2.15±0.04a	2.25±0.07a	1.06±0.09c	1.44±0.00b
Natural habitat	-	-	-	-	-	-	-	-	1.75±0.13ab	2.02±0.07a	1.79±0.08a	2.75±0.04a

Means that followed by different letters are significantly different at P < 0.05 using LSD tests.

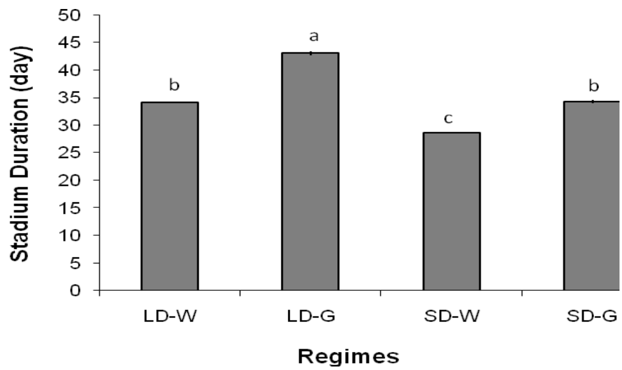

Figure 1. Effect of diet and photoperiod on stadium duration of Sunn pest.
LD-W: Long day condition and wheat grain diet, SD-W: Short day condition and wheat grain diet, LD-G: Long day condition and wheat plant diet, SD-G: Short day condition and wheat plant diet. Means that followed by different letters are significantly different at P < 0.05 using LSD tests.

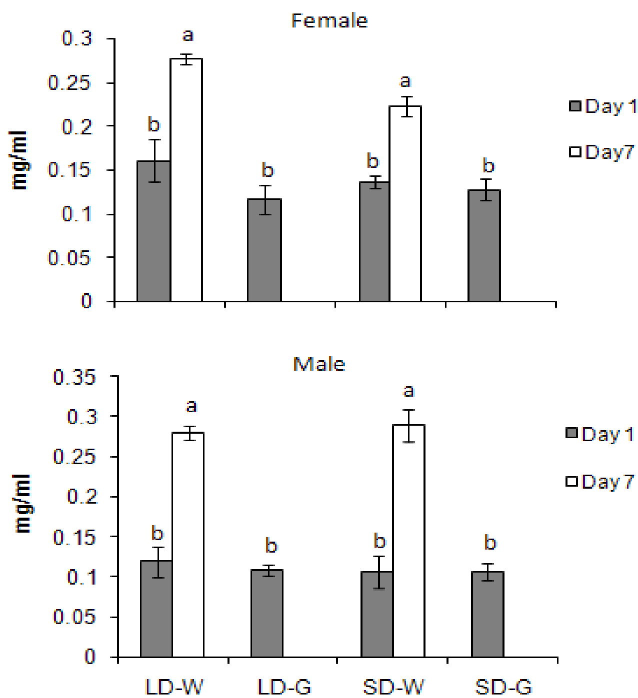

Figure 2. Effect of diet and photoperiod on haemolymph protein concentration of the Sunn pest.
LD-W: Long day condition and wheat grain diet, SD-W: Short day condition and wheat grain diet, LD-G: Long day condition and wheat plant diet, SD-G: Short day condition and wheat plant diet. Means that followed by different letters are significantly different at P < 0.05 using LSD tests. Protein concentration was not determined at day seven when insect offered wheat growing plant.

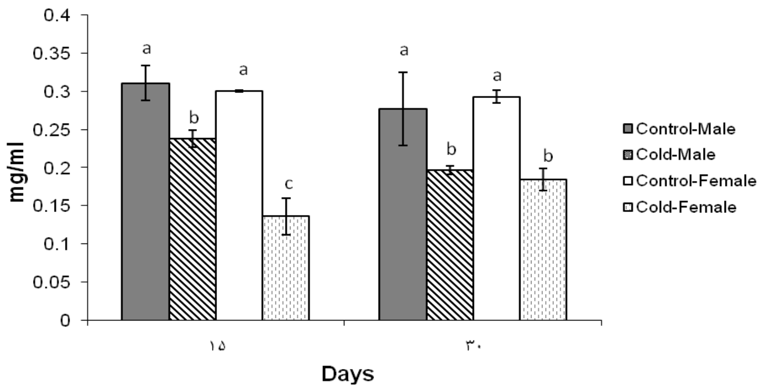

Figure 3. Effect of cold on haemolymph protein concentration of Sunn pest.
Means that followed by different letters are significantly different at $P < 0.05$ using LSD tests.