

# Wheat cultivars affecting life history and digestive amylolytic activity of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae)

E. Borzoui and B. Naseri\*

Department of Plant Protection, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil, Iran

## Abstract

The life history and digestive  $\alpha$ -amylase activity of the Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) were studied on six wheat cultivars (Arg, Bam, Nai 60, Pishtaz, Sepahan and Shanghai) at  $25 \pm 1^\circ\text{C}$ , relative humidity of  $65 \pm 5\%$  and a photoperiod of 16:8 (L:D) h. A delay in the developmental time of *S. cerealella* immature stages was detected when larvae were fed on cultivar Sepahan. The maximum survival rate of immature stages was seen on cultivar Bam ( $93.33 \pm 2.10\%$ ), and the minimum rates were on cultivars Nai 60 ( $54.66 \pm 2.49\%$ ) and Sepahan ( $49.33 \pm 4.52\%$ ). The highest realized fecundity and fertility were recorded for females which came from larvae fed on cultivar Bam ( $93.30 \pm 2.10$  eggs/female and  $91.90 \pm 3.10\%$ , respectively); and the lowest ones were observed for females which came from larvae fed on cultivar Sepahan ( $49.30 \pm 4.50$  eggs/female and  $67.4 \pm 11.1\%$ , respectively). The heaviest male and female weights of *S. cerealella* were observed on cultivar Bam ( $2.97 \pm 0.02$  and  $4.80 \pm 0.01$  mg, respectively). The highest amylolytic activity of the fourth instar was detected on cultivar Bam ( $0.89 \pm 0.04$  mg maltose  $\text{min}^{-1}$ ), which had the maximum mean hundred-wheat weight ( $5.92 \pm 0.19$  g). One  $\alpha$ -amylase isozyme was detected in the midgut extracts from the fourth instar larvae fed on different wheat cultivars, and the highest intensity was found in larvae fed on cultivar Bam. Correlation analyses showed that very high correlations existed between the immature period, fecundity and fertility on one side and inhibition of  $\alpha$ -amylase, soluble starch content and hundred-wheat weight on the other. According to the obtained results, cultivar Sepahan is an unfavorable host for the feeding and development of *S. cerealella*.

**Keywords:** Angoumois grain moth, life span, survival rate, nutritional physiology

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## Introduction

The Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), is an important pest of wheat (*Triticum aestivum* L.) and other grains throughout the world

(Weston & Rattlingourd, 1999; Shukle & Wu, 2003). The larvae of *S. cerealella* cause serious damage by feeding on kernels and producing feces (Khattak *et al.*, 1996; Ahmed *et al.*, 2002; Throne & Weaver, 2013). However, physical and chemical characteristics of host grains can influence the feeding potential of *S. cerealella* (Ahmed & Raza, 2010).

Pesticides have been the primary tools for controlling grain pests (Hosseiniaveh *et al.*, 2007); however, the use of these pesticides is limited because of their adverse effects on humans and the environment. It is now necessary to develop more environmentally benign alternatives for controlling

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\*Author for correspondence  
 Phone: +98 9142857597  
 Fax: +98 4533512204  
 E-mail: [bnaseri@uma.ac.ir](mailto:bnaseri@uma.ac.ir)

stored-products pests. Among available options, host-plant resistance offers one of the most successful preservation methods for many agricultural and food crops (Hagstrum & Subramanyam, 1996; Jayas & Jeyamkondan, 2002; Hashem, *et al.*, 2012).

The life history of insects has been reported to be affected by different factors. Various macronutrients especially protein and carbohydrate content, have a significant effect on the life history of insect pests (Ohmart, 1991, Hanks *et al.*, 1993; El Atta, 2000). There are comprehensive studies on the life history of *S. cerealella* fed on different grain cultivars (Khan *et al.*, 2010; Saikia *et al.*, 2014). For example, Consoli & Filho (1995) studied the effects of different corn genotypes on the development and reproduction of *S. cerealella* and reported that the longest larval period and the lowest fecundity was on genotype Shrunhen. Shafique *et al.* (2006) examined the effect of different wheat cultivars on the egg hatch of *S. cerealella* and showed that Punjab-96 and Chenab-99 were the least suitable wheat cultivars for this pest.

Invertebrate herbivores are faced with nutritional challenges, such as harmful secondary metabolites, proteinaceous inhibitors and imbalanced nutrition, and these affect the optimal growth, development, reproductive potential and population dynamic of insects (Awmack & Leather, 2002; Babic *et al.*, 2008). These herbivores regulate nutrients to increase growth performance (Simpson *et al.*, 2004; Behmer, 2009). Post-ingestive regulation is one of the strategies for dealing with nutritional imbalances and proteinaceous inhibitors in the restricted range of available foods (Kotkar *et al.*, 2009).

Alpha-amylases are widespread in nature, being found in animals, microorganisms and plants (Terashima & Katoh, 1996; Franco *et al.*, 2000). *S. cerealella* lives on a starch-rich diet and relies on the effectiveness of its  $\alpha$ -amylases for development. The  $\alpha$ -Amylase activity has been described in several stored-product pests: *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) (Mendiola-Olaya *et al.*, 2000); *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) (Cinco-Moroyoqui *et al.*, 2006); *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) (Bouayad *et al.*, 2008); *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Pytelkova *et al.*, 2009); *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) (Dastranj *et al.*, 2013); *Trogoderma granarium* Everts (Coleoptera: Dermestidae) (Hosseiniaveh *et al.*, 2007; Borzoui *et al.*, 2015). Despite the economic importance of *S. cerealella* on wheat grains, there is no published information regarding the digestive amylolytic activity of this pest. Therefore, this study is the first attempt to investigate the digestive  $\alpha$ -amylase activity of *S. cerealella* on different wheat cultivars.

The goal of this research was to study the biological characteristics and midgut  $\alpha$ -amylase activity of *S. cerealella* larvae when fed on different wheat cultivars. An understanding of how digestive  $\alpha$ -amylase function and the nutritional physiology of *S. cerealella* on wheat cultivars, and determining the factors affecting its life history is required when developing methods of pest management, such as the application of resistant cultivars to control the pest.

## Materials and methods

### Wheat sources

Seed of six wheat cultivars including Arg, Bam, Nai 60, Pishtaz, Sepahan and Shanghai were obtained from the Plant and Seed Improvement Research Institute (Karaj, Iran).

### Insect rearing

The adults of *S. cerealella* were collected from stored maize seeds in Ardabil, Iran. Their larvae were fed on maize seeds and maintained at  $25 \pm 1^\circ\text{C}$ , relative humidity of  $65 \pm 5\%$ , and a photoperiod of 16:8 (L:D) h, as described by Throne & Weaver (2013). After colonization of *S. cerealella*, the insects tested on different wheat cultivars were reared for three generations on the same cultivars. Moisture content of wheat cultivars was measured using the hot air oven method (AOAC, 1984) and varied from 10.2 to 11.8%.

### Developmental time and survival rate of *S. cerealella*

A quantity of 500 g of each wheat cultivar was held in the experimental room for 48 h before the experiments. One hundred newly deposited eggs (within 24 h) of *S. cerealella* were individually transferred into Petri dishes (diameter 6 cm, depth 1 cm), containing one seed of each wheat cultivar. Petri dishes were visited daily, and the duration of immature stages (from egg to emergence of adult), immature survival and adult longevity were recorded.

### Realized fecundity and egg fertility

Newly emerged adults (one male and one female) were transferred to plastic vials (diameter 5 cm, depth 10 cm) to evaluate realized fecundity. Black paper sheets were used as the substrate of deposited eggs (Ellington, 1930). The paper sheets were removed daily and the number of deposited eggs was recorded until the female's death. The eggs were maintained for 12 days to estimate the percentage of hatched eggs (fertility).

### Weight of adults

The newly deposited eggs (within 24 h) of *S. cerealella* were individually transferred into glass dishes (diameter 15 cm, depth 20 cm), containing 200 g of each wheat cultivar. These glass dishes were held in the experimental room until adults emerged. The male (25–41 replicates) and female (25–49 replicates) adults emerged from larvae reared on each wheat cultivar were weighed separately.

### Extraction of the proteinaceous inhibitors

The proteinaceous inhibitor content of the tested wheat cultivars was extracted based on the methods of Baker (1987) and Melo *et al.* (1999). Separately, each wheat cultivar was powdered thoroughly, and then 30 g of powdered wheat was mixed with a solution of 0.1 M NaCl and stirred for 1.5 h, followed by centrifugation at 8000 g for 30 min. The supernatant was taken and heated at  $70^\circ\text{C}$  for 30 min to inactivate endogenous enzymes. Proteins were concentrated using a saturation of 70% ammonium sulfate followed by centrifugation at 8000 g for 30 min at  $4^\circ\text{C}$ . Fractionation of supernatant was done using 20, 40, 60 and 80% ammonium sulfate saturations. Pellets obtained from 80% saturation of ammonium sulfate were dissolved in the phosphate buffer (0.02 M, pH 7) and dialyzed against the same buffer overnight. This dialyzed solution was used as a source of inhibitor in enzymatic assays.

Protein concentration of inhibitors of the tested wheat cultivars was measured using bovine serum albumin (Roche Co., Germany) (Bradford, 1976).

### Enzymes preparation

The fourth instar larvae of *S. cerealella* fed on each cultivar were carefully dissected in pre-cooled distilled water under stereomicroscope (Stemi SV6 ZEISS, Germany) (Borzoui & Bandani, 2013). Midguts were separated and homogenized on ice using a pre-cooled homogenizer (Teflon pestle). The homogenates were centrifuged at 15,000 g for 15 min at 4°C. The supernatants were pooled and stored in aliquots at -20°C as an enzyme source for subsequent use. For inhibition experiment, the enzyme extract prepared from *S. cerealella* larvae fed on maize was used.

### Effect of pH on digestive amylolytic activity

The effect of pH on  $\alpha$ -amylase activity was examined using  $\alpha$ -amylase extracted from fourth instar larvae fed on maize. Optimal pH for  $\alpha$ -amylase activity was determined using universal buffer (0.02 M) (Hosseinkhani & Nemat-Gorgani, 2003; Borzoui & Bandani, 2013) with pH set at 5–12.

### Amylolytic activity assay

The dinitrosalicylic acid (DNS) method (Bernfeld, 1955), with 1% soluble starch (Sigma Chemical Co., St Louis, USA) as substrate at the optimal pH was used to assay the digestive amylolytic activity of *S. cerealella* larvae fed on different wheat cultivars. Twenty microliters of the enzyme extracted from 30 midguts were incubated with 500  $\mu$ l of glycine-NaOH buffer (pH 11.5) (Borzoui & Bandani, 2013) and 40  $\mu$ l of soluble starch for 30 min at 37°C. The reaction was stopped by adding 100  $\mu$ l of DNS (Sigma Chemical Co., St Louis, USA) and heating in boiling water for 10 min. The absorbance of the mixture was read at 540 nm after being cooled on ice. One unit of  $\alpha$ -amylase activity was defined as the quantity of enzyme required to produce 1 mg maltose at 37°C min<sup>-1</sup>. A standard curve of absorbance against the amount of maltose (Sigma Chemical Co., St Louis, USA) released was constructed to enable its calculation during  $\alpha$ -amylase assays. A control containing no  $\alpha$ -amylase extract with substrate was run simultaneously with the reaction mixture.

### In-gel amylolytic activity assay

The amylolytic activity of *S. cerealella* in the gel was detected using the method of Kazzazi *et al.* (2005). Briefly, polyacrylamide gel electrophoresis (PAGE) was performed in a 10% (w/v) separating gel and a 4% stacking gel with 0.05% SDS. Twenty microliters of the enzyme extracts (from 30 larval midguts) were mixed with an equal volume of zymogram sample buffer. Thirty five microliters of each sample were loaded on each well of zymogram gel. Electrophoresis was run until the blue dye reached the bottom of the gel. The gel was then rinsed with distilled water and left in a solution of 1% (v/v) Triton X-100 for 15 min. Thereafter, the gel was taken and put in a solution of glycine-NaOH (pH 11.5) containing 1% starch solution, 2 mM CaCl<sub>2</sub> and 10 mM NaCl for 1.5 h at 37°C. To stop the reaction and stain the un-reacted starch background, the gel was treated with a solution of 1.3% I<sub>2</sub> and 3% KI. Finally, light bands of  $\alpha$ -amylase activities appeared against a dark background.

### Inhibition of *S. cerealella* $\alpha$ -amylases by wheat cultivars

In this experiment, 1.5 mg proteinaceous extract of each cultivar was incubated (30 min at 37°C) with amylase extracted from fourth instar larvae fed on maize (Baker, 1987; Melo *et al.*, 1999). For the control group, we used the same enzyme extract of fourth instar larvae without inhibitor. The procedure for the  $\alpha$ -amylase assay was conducted as noted in the 'Amylolytic activity assay' section. The percentage of  $\alpha$ -amylase inhibition was calculated according to Borzoui & Bandani (2013).

### Weight of tested wheat cultivars

The weight of the grains of tested wheat cultivars was measured as the mean hundred-wheat weight (Fouad *et al.*, 2013). Five hundred seeds of each cultivar (in five replicates, each including 100 seeds) were randomly collected and weighed using an electronic balance.

### Starch determination of wheat cultivars

The starch content of tested cultivars was quantified by the method of Bernfeld (1955) using starch as a standard. Briefly, each wheat cultivar was powdered thoroughly, and then 200 mg of each cultivar were homogenized in 35 ml of distilled water and heated to boiling point. One hundred microliters of each sample was added to 2.5 ml of iodine reagent (0.02% I<sub>2</sub> and 0.2% KI), and the absorbance was read at 580 nm.

### Statistical analysis

Before analysis, all the data were examined for normality using Kolmogorov-Smirnov test (PROC GLM; SAS Institute, 2003). Since the data were normally distributed, no data transformation was conducted. Life history and digestive  $\alpha$ -amylase activity of *S. cerealella* were analyzed, based on a completely randomized design, by one-way analysis of variance (ANOVA) using SAS ver.9.2 program (PROC GLM; SAS Institute, 2003). Finally, the statistical differences among the means were appraised using the least significant difference (LSD) test at  $\alpha = 0.05$ . Correlation analysis of the examined biological and physiological characteristics of *S. cerealella* fed on different wheat cultivars with inhibition of  $\alpha$ -amylase, soluble starch content and hundred-wheat weight was performed using SPSS 16.0.

## Results

### Developmental time and survival rate

The results of the effect of various wheat cultivars on developmental time of *S. cerealella* are given in table 1. The shortest developmental time of immature stages (from egg to emergence of adult) ( $F = 85.69$ ;  $df = 5, 407$ ;  $P < 0.0001$ ) was on cultivar Bam ( $23.65 \pm 0.15$  days), and the longest time was on cultivar Sepahan ( $32.63 \pm 0.66$  days). Different cultivars showed a significant effect on the longevity of male ( $F = 23.43$ ;  $df = 5, 195$ ;  $P < 0.0001$ ) and female ( $F = 33.48$ ;  $df = 5, 206$ ;  $P < 0.0001$ ) of *S. cerealella*, which was shortest on cultivar Sepahan ( $5.96 \pm 0.20$  and  $6.80 \pm 0.20$  days, respectively), and longest on cultivar Bam ( $8.14 \pm 0.10$  and  $9.38 \pm 0.11$  days, respectively) (table 1).

Table 1. Mean ( $\pm$ SE) duration (days) of immature stages and longevity of *S. cerealella* fed on different wheat cultivars.

Wheat cultivars	Developmental period									
	$n^1$	Egg incubation	$n$	Larval and pupal period	$n$	Immature period	$n$	Male longevity	$n$	Female longevity
Arg	90	3.23 $\pm$ 0.06 d	81	22.01 $\pm$ 0.28 d	81	25.22 $\pm$ 0.30 d	40	7.52 $\pm$ 0.13 b	41	8.75 $\pm$ 0.12 b
Bam	95	2.91 $\pm$ 0.07 e	91	20.74 $\pm$ 0.17 e	91	23.65 $\pm$ 0.15 e	41	8.14 $\pm$ 0.10 a	49	9.38 $\pm$ 0.11 a
Nai 60	82	3.92 $\pm$ 0.09 b	59	25.18 $\pm$ 0.37 b	59	29.13 $\pm$ 0.40 b	27	6.70 $\pm$ 0.17 c	32	7.53 $\pm$ 0.17 d
Pishtaz	83	3.59 $\pm$ 0.07 c	65	23.56 $\pm$ 0.23 c	65	27.18 $\pm$ 0.22 c	32	7.03 $\pm$ 0.15 c	33	7.96 $\pm$ 0.15 cd
Sepahan	75	4.29 $\pm$ 0.11 a	49	28.16 $\pm$ 0.69 a	49	32.63 $\pm$ 0.66 a	25	5.96 $\pm$ 0.20 d	25	6.80 $\pm$ 0.20 e
Shanghai	83	3.53 $\pm$ 0.07 c	68	23.47 $\pm$ 0.25 c	68	26.95 $\pm$ 0.26 c	36	7.13 $\pm$ 0.14 bc	32	8.03 $\pm$ 0.19 c

The means followed by different letters in the same column are significantly different (LSD,  $P < 0.05$ ).

<sup>1</sup>The  $n$  value shows the sample size for each parameter.

The survival rates of *S. cerealella* immature stages on six wheat cultivars are shown in fig. 1. The lowest survival rate ( $F = 28.09$ ;  $df = 5, 24$ ;  $P < 0.0001$ ) of immature stages was observed on cultivars Nai 60 (54.66  $\pm$  2.49%) and Sepahan (49.33  $\pm$  4.52%), and the highest survival rate was seen on cultivar Bam (93.33  $\pm$  2.10%) (fig. 1).

#### Realized fecundity and egg fertility

The results of realized fecundity and egg fertility of *S. cerealella* are shown in table 2. The highest realized fecundity ( $F = 136.33$ ;  $df = 5, 206$ ;  $P < 0.0001$ ) and egg fertility ( $F = 53.79$ ;  $df = 5, 206$ ;  $P < 0.0001$ ) of *S. cerealella* were recorded for females developed from larvae reared on cultivar Bam (93.30  $\pm$  2.10 eggs/female and 91.90  $\pm$  3.10%, respectively), and the lowest values of these parameters were recorded on cultivar Sepahan (49.30  $\pm$  4.50 eggs/female and 67.4  $\pm$  11.1%, respectively) (table 2).

#### Weight of adults

The results of adults' weight of *S. cerealella* on different wheat cultivars are shown in fig. 2. The male adults of *S. cerealella* which came from larvae fed on cultivar Bam were heavier (2.97  $\pm$  0.02 mg) than those reared on any other cultivar ( $F = 176.40$ ;  $df = 5, 196$ ;  $P < 0.0001$ ). In addition, females ( $F = 297.56$ ;  $df = 5, 206$ ;  $P < 0.0001$ ) which came from larvae reared on cultivar Bam showed the highest weight (4.80  $\pm$  0.01 mg) as compared with other cultivars tested (fig. 2). By contrast, the lowest weight of male adults of *S. cerealella* was seen in the insects that came from larvae reared on cultivars Nai 60 (2.17  $\pm$  0.03 mg) and Sepahan (2.15  $\pm$  0.03 mg). Moreover, the lowest weight of female adults was seen in the insects that came from larvae fed on cultivar Sepahan (3.65  $\pm$  0.03 mg).

#### Optimal pH of $\alpha$ -amylase

The effect of pH on amyolytic activity of the midgut extract from the fourth instar larvae of *S. cerealella* is presented in fig. 3 ( $F = 201.22$ ;  $df = 14, 30$ ;  $P < 0.0001$ ). Amyolytic activity with starch as substrate occurred over an alkaline pH range (pH 9–11), with maximum activity at pH 11.5. In acidic conditions (pH 5–7), little activity (under 20% of maximum activity) was observed. In highly alkaline conditions (pH 12), approximately 70% of maximum activity was recorded.

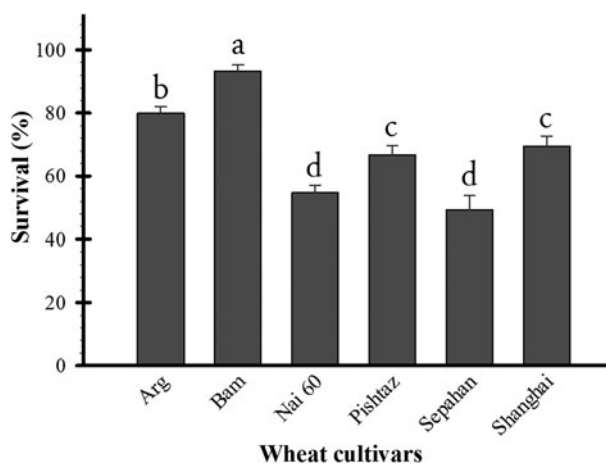


Fig. 1. Mean ( $\pm$ SE) percentage survival of *S. cerealella* immature stages ( $n = 49$ –91) on different wheat cultivars. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

#### Enzyme assay

Amyolytic activity for the fourth instar larvae of *S. cerealella* feeding on different wheat cultivars is shown in fig. 4 ( $F = 19.60$ ;  $df = 5, 12$ ;  $P < 0.0001$ ). The fourth instar larvae reared on cultivar Bam had the highest level of amyolytic activity (0.89  $\pm$  0.04 mg maltose  $\text{min}^{-1}$ ). By contrast, the lowest levels of amyolytic activity were seen on cultivars Nai 60 and Sepahan (0.42  $\pm$  0.03 and 0.39  $\pm$  0.04 mg maltose  $\text{min}^{-1}$ , respectively).

#### In-gel enzyme analysis

Amyolytic activity pattern of the fourth instar larvae of *S. cerealella* feeding on different wheat cultivars is shown in fig. 5. One prominent  $\alpha$ -amylase isozyme was detected in the midgut extracts of larvae fed on the tested wheat cultivars, but, the intensity of individual  $\alpha$ -amylase isozymes varied by cultivars. According to the results of this study, larvae reared on cultivar Sepahan showed a weak intensity of amyolytic band as compared with those reared on cultivars Bam, Arg and Shanghai. Furthermore, larvae fed on cultivar Bam had a stronger intensity than those fed on the other cultivars (fig. 5).

Table 2. Mean ( $\pm$ SE) realized fecundity (eggs laid per female) and egg fertility (percentage of hatched eggs per female) of *S. cerealella* fed on different wheat cultivars.

Wheat cultivars	$n^1$	Fecundity	$n$	Fertility (%)
Arg	41	80.0 $\pm$ 2.1 b	41	84.6 $\pm$ 6.7 b
Bam	49	93.3 $\pm$ 2.1 a	49	91.9 $\pm$ 3.1 a
Nai 60	32	54.6 $\pm$ 2.4 d	32	74.5 $\pm$ 7.7 d
Pishtaz	33	66.6 $\pm$ 2.9 c	33	78.3 $\pm$ 6.0 c
Sepahan	25	49.3 $\pm$ 4.5 e	25	67.4 $\pm$ 11.1 e
Shanghai	32	69.3 $\pm$ 3.3 c	32	79.1 $\pm$ 6.5 c

The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

<sup>1</sup>The  $n$  value shows the sample size for each parameter.

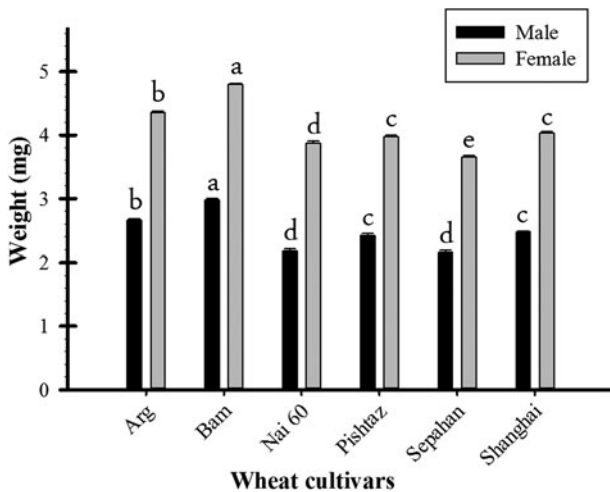


Fig. 2. Mean ( $\pm$ SE) weight of male ( $n = 25-41$ ) and female ( $n = 25-49$ ) adults of *S. cerealella* fed on different wheat cultivars. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

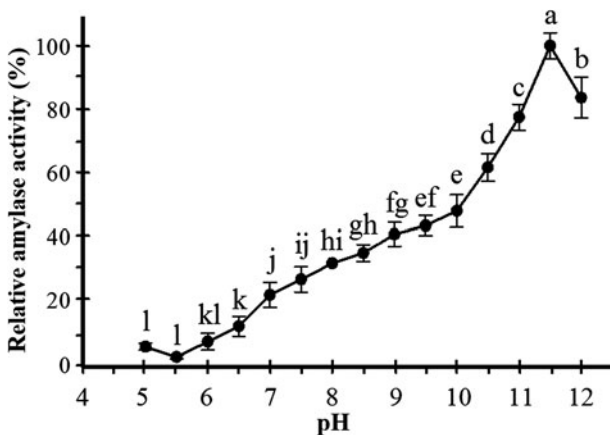


Fig. 3. Effect of pH on the amyolytic activity ( $n = 3$ ) of *S. cerealella*. Activity was determined using universal buffer. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

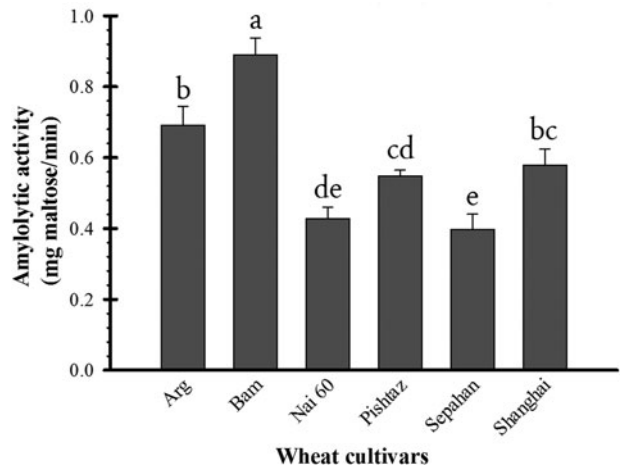


Fig. 4. Mean ( $\pm$ SE)  $\alpha$ -amylase activity of midgut extracts ( $n = 3$ ) from *S. cerealella* larvae fed on different wheat cultivars. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

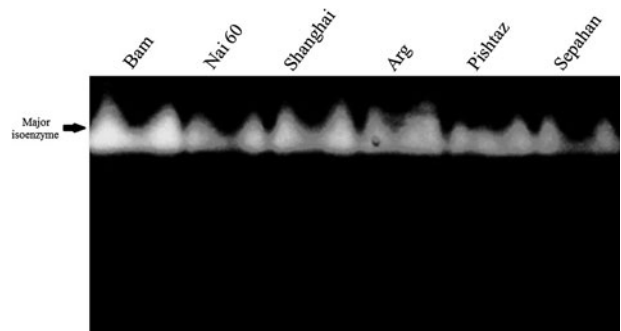


Fig. 5. In-gel visualization of  $\alpha$ -amylase activity isozyme in the midgut of *S. cerealella* larvae fed on six wheat cultivars. One band of the  $\alpha$ -amylase activity was observed in each sample, and this band was designated as a major isoenzyme.

#### Inhibition of $\alpha$ -amylases by wheat cultivars

The effect of inhibitor extracted from wheat cultivars on digestive  $\alpha$ -amylase activity of *S. cerealella* is shown in fig. 6 ( $F = 12.93$ ;  $df = 5, 12$ ;  $P < 0.0001$ ). Maximum inhibition of  $\alpha$ -amylase activity was estimated by incubating midgut extracts from the fourth instar larvae with proteinaceous extract of cultivars Sepahan and Nai 60 ( $85.32 \pm 5.90$  and  $75.91 \pm 1.83\%$ , respectively).

#### Dietary starch content

Dietary starch content of different wheat cultivars used for feeding assays of *S. cerealella* is shown in fig. 7. Cultivar Bam had the highest starch content ( $413.66 \pm 5.81$  mg  $g^{-1}$  fresh weight) and cultivar Sepahan had the lowest ( $363.33 \pm 12.77$  mg  $g^{-1}$  fresh weight) ( $F = 4.75$ ;  $df = 5, 12$ ;  $P < 0.0001$ ). However, there were no significant differences in starch content among cultivars Bam, Nai 60, Arg and Pishtaz.

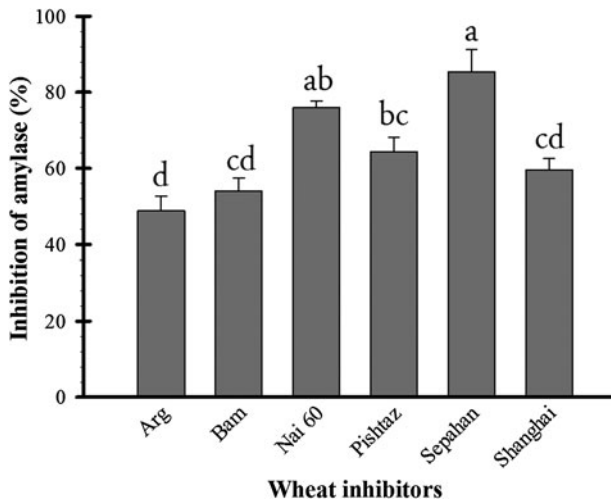


Fig. 6. Mean ( $\pm$ SE) percentage inhibition of amylolytic activity of *S. cerealella* larval midgut ( $n = 3$ ) by crude inhibitor extracted from different wheat cultivars. Enzyme and inhibitor were incubated for 30 min prior to the addition of starch to measure enzymatic activity. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

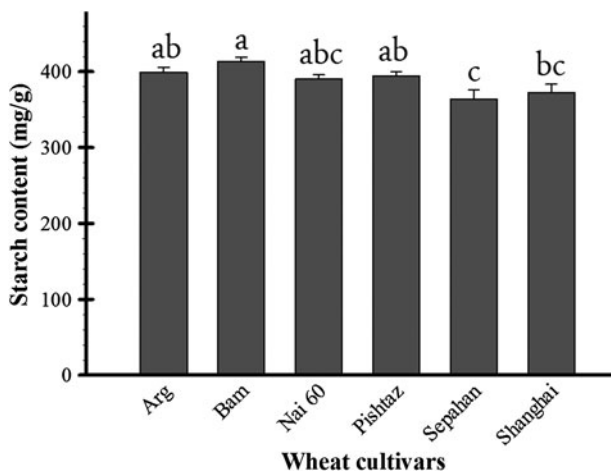


Fig. 7. Soluble starch content of different wheat cultivars used for feeding of *S. cerealella* larvae. Each point is average of three replications. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

#### Weight of tested wheat cultivars

Fig. 8 shows the mean hundred-wheat weight of tested cultivars ( $F = 12.93$ ;  $df = 5, 12$ ;  $P < 0.0001$ ). The mean hundred-wheat weight was significantly heavier ( $5.92 \pm 0.19$  g) in cultivar Bam than in the other cultivars. By contrast, the weight of cultivar Sepahan was the lightest ( $4.02 \pm 0.19$  g) compared with the other cultivars.

#### Correlation analysis

The analysis of correlation coefficients of the examined biological and physiological characteristics of *S. cerealella* fed on

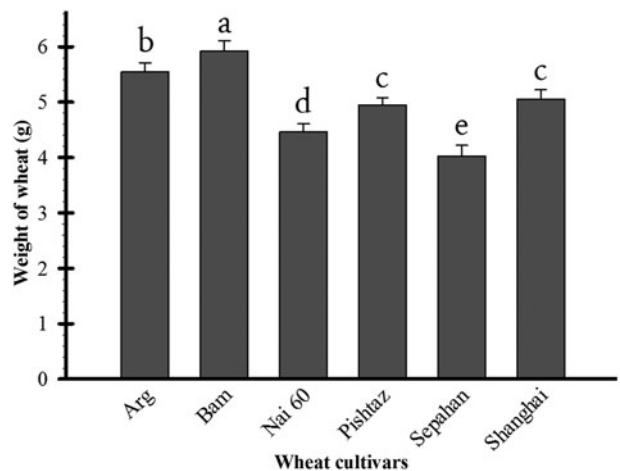


Fig. 8. The mean hundred-wheat weight of different tested wheat cultivars. The means followed by different letters are significantly different (LSD,  $P < 0.05$ ).

different wheat cultivars with inhibition of  $\alpha$ -amylase, soluble starch content and hundred-wheat weight is shown in table 3. The results of this study showed that very high correlations existed between the immature period, fecundity and fertility on one side and inhibition of  $\alpha$ -amylase, soluble starch content and hundred-wheat weight on the other. Very high positive correlations were also found between fecundity ( $r = 0.876$  and  $0.989$ , respectively) and fertility ( $r = 0.864$  and  $0.990$ , respectively) and soluble starch content and hundred-wheat weight. Moreover, a negative correlation was found between fecundity and fertility ( $r = -0.896$  and  $-0.896$ , respectively) and inhibition of  $\alpha$ -amylase. The immature period exhibited a negative correlation with soluble starch content and hundred-wheat weight ( $r = -0.839$  and  $-0.983$ , respectively) and a positive correlation with inhibition of  $\alpha$ -amylase ( $r = 0.946$ ). Correlation coefficients showed that amylolytic activity exhibited a positive correlation with inhibition of  $\alpha$ -amylase ( $r = 0.842$ ) and a very high positive correlation with hundred-wheat weight ( $r = 0.967$ ) (table 3).

## Discussion

In this study, the biological and physiological properties of *S. cerealella* were influenced when exposed to different wheat cultivars. *S. cerealella* required a longer time to complete its immature stages when reared on cultivar Sepahan than when reared on the other cultivars. The quality and quantity of food have a direct effect on basic aspects of the biology of insects (Pashley *et al.*, 1995; Bentancourt *et al.*, 2003; Bong *et al.*, 2008). Variations in the duration of immature stages of *S. cerealella* might be attributed to the differences in the macronutrients (especially starch), inhibitors and weight of tested wheat cultivars.

The results regarding the incubation period of *S. cerealella* agreed with those achieved by Saikia *et al.* (2014), who reported incubation periods of  $2.8 \pm 0.8$  and  $3.3 \pm 0.5$  days for *S. cerealella* reared on maize and rice, respectively. The developmental time of whole larval and pupal stages of *S. cerealella*, in this study, is not in agreement with the findings of Shukle & Wu (2003), who noted that the developmental time of the

Table 3. Correlation coefficients ( $r$ ) of some biological and physiological characteristics of *S. cerealella* fed on different wheat cultivars with inhibition of  $\alpha$ -amylase, soluble starch content and hundred-wheat weight.

Parameter	Inhibition of $\alpha$ -amylase		Soluble starch content		Hundred-wheat weight	
	$r$	$P_{\text{value}}$	$r$	$P_{\text{value}}$	$r$	$P_{\text{value}}$
Egg incubation	0.937	0.006	-0.828	0.042	-0.988	0.000
Larval and pupal period	0.944	0.005	-0.843	0.035	-0.981	0.001
Immature period	0.946	0.004	-0.839	0.037	-0.983	0.000
Male longevity	0.463	0.355	-0.126	0.812	-0.367	0.475
Female longevity	0.546	0.262	-0.186	0.725	-0.455	0.365
Fecundity	-0.896	0.016	0.876	0.022	0.989	0.000
Fertility	-0.896	0.016	0.864	0.027	0.990	0.000
Survival	-0.900	0.015	0.800	0.056	0.990	0.000
Weight of male	0.557	0.251	-0.048	0.928	-0.444	0.377
Weight of female	0.516	0.194	-0.193	0.715	-0.530	0.280
Amylolytic activity	0.842	0.035	0.792	0.060	0.967	0.002

whole larval and pupal stages of *S. cerealella* was  $46.6 \pm 5.6$  days on wheat seeds mixed with Brewer's yeast. Such discrepancy might be attributed to either genetic differences in populations or variations in experimental conditions and cultivars used for feeding of this pest.

In agreement with previous research (Consoli & Filho, 1995; Ashamo, 2010), the results of this study showed that tested wheat cultivars had significant effects on the longevity of *S. cerealella* adults. The shortest adult longevity of *S. cerealella* on cultivar Sepahan suggested that this cultivar did not contain all of the ingredients necessary for the optimal development of *S. cerealella* larvae, which led to the emergence of adults with shorter longevity.

The reduced survival rates of this pest found on cultivars Nai 60 and Sepahan might be attributed to their negative correlation ( $r = -0.900$ ) with inhibition of  $\alpha$ -amylase (table 3). The range of survival rates of *S. cerealella*, in the current study, is in agreement with those reported for *S. cerealella* reared on paddy varieties (Ashamo, 2010) and corn genotypes (Consoli & Filho, 1995). Gatehouse & Boulter (1983) suggested that the level of inhibitors in legumes is related to the observed resistance to pests. In this study, the digestive  $\alpha$ -amylase activities of *S. cerealella* larvae reared on different wheat cultivars were affected by the proteinaceous extract of these cultivars (fig. 6) that led to the reduced growth and increased mortality of this insect on some tested cultivars. Studies on plant inhibitors and insect digestive enzymes indicated that insect enzymes responded to exposed inhibitors by changing the complement of midgut proteinases (Dunse *et al.*, 2010; Lomate & Hivrale, 2011). In some insects, the produced proteinases are able to degrade inhibitors, and reduce the adverse effect on pest growth and development (Bown *et al.*, 1997; Giri *et al.*, 1998). There was also a positive correlation between food availability (weight of each cultivar) and survival of the pest (table 3). An increased food availability implies a change in the activity of digestive enzymes to ensure proper nutritional requirements (Kotkar *et al.*, 2009; Borzoui *et al.*, 2015).

The performance of lepidopteran adults can directly be influenced by larval food (Nay & Perring, 2008; Sarate *et al.*, 2012). The results of this study showed a small body size in adults reared on cultivar Sepahan (an unsuitable host); however, no significant correlation was found between the adult weight of *S. cerealella* and the dietary factors of tested cultivars (table 3). This result is consistent with the data obtained by Ahmed & Raza (2010), who reported a significant difference

in the adult weight of *S. cerealella* fed on various maize varieties. Furthermore, the realized fecundity and egg fertility of *S. cerealella* was higher in females fed on cultivar Bam because of the higher nutritional value of this cultivar, particularly in relation to its weight, starch content and level of inhibitors. Similar results were observed by Shobana *et al.* (2010), with *Papilio polytes* Linnaeus (Lepidoptera: Papilionidae) reared on a nutrient-rich diet.

The results of the mean hundred-wheat weight of each tested cultivar showed that the physical characteristics of wheat cultivars had an apparent effect on the biological and physiological aspects of *S. cerealella*. Shazali (1987) showed that *S. cerealella* consumed significantly more food in the larger grain type than in the smaller one. In the present study, the direct correlations (table 3) were clear, even though there were differences in life history, realized fecundity, egg fertility and  $\alpha$ -amylase activity of *S. cerealella* among the large and small size of the wheat cultivars (of viewpoint the mean hundred-wheat weight). The insects reared on cultivar Bam (with the highest weight) completed their immature stages faster than those reared on the other cultivars (table 1, fig. 8). Moreover, realized fecundity and egg fertility were elevated with increase in the weight of wheat cultivars (tables 2 and 3). Similarly, an increase in the level of  $\alpha$ -amylase activity was obtained with increase in the weight of wheat cultivars (table 3). It seems that when seed weight is increased, more food is available for the feeding of *S. cerealella* larvae; therefore, the larvae elevated  $\alpha$ -amylase levels to get more nutrients. Furthermore, the larvae could have been used these optimal food conditions for optimal fitness. This finding is also supported by previous studies (Behmer, 2009; Kotkar *et al.*, 2009; Karasov *et al.*, 2011; Borzoui *et al.*, 2015) that have stated that the kind and level of digestive enzymes in many insects can be largely explained by the interaction between diet macronutrients and insect food needs, which ultimately affect the life cycle of these insects.

This study is the first attempt to characterize amylolytic activity in larvae of *S. cerealella*. High amylolytic activity was found in the midgut of larvae of *S. cerealella* as reported for other lepidopteran grain pests (Bouayad *et al.*, 2008; Pytelkova *et al.*, 2009). The pH is a key factor affecting biochemical reactions of digestive enzymes. The results of this study showed that the  $\alpha$ -amylase enzyme was active over an alkaline pH range (pH 9–12). According to the results of Tabatabaei *et al.* (2011), the  $\alpha$ -amylase enzyme from

*Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) was active in the pH range of 7–11.

Results from the current study on the digestive  $\alpha$ -amylase of *S. cerealella* showed that this lepidopteran pest replies to different wheat cultivars by changing digestive enzymes. Correlation analyses and comparisons of  $\alpha$ -amylase activities of the fourth instar larvae fed on tested cultivars suggested that factors found in the cultivars (weight and  $\alpha$ -amylase inhibitors) influenced the  $\alpha$ -amylase activity. This result coincides with the outcomes reported by Harsulkar *et al.* (1999) and Kotkar *et al.* (2009), for *H. armigera*. However, Bouayad *et al.* (2008) reported that the level of  $\alpha$ -amylase activity in larvae of *P. interpunctella* was dependent upon the dietary carbohydrate source.

In this study, a difference in the expression level of  $\alpha$ -amylase isozyme was detected in the PAGE. Considering the relationship between the intensity of  $\alpha$ -amylase band and the hundred-wheat weight of each cultivar, it seems that there is a mechanism to precisely discover the diet content and adjust the level of this major digestive enzyme (Behmer, 2009; Kotkar *et al.*, 2009; Sarate *et al.*, 2012). Silva *et al.* (2001) showed that the  $\alpha$ -amylase gene was regulated according to the amount of starch in the diet. Interestingly, although starch contents were not significantly different between cultivars Arg and Bam (fig. 7), the midgut  $\alpha$ -amylase from larvae fed on cultivar Bam was higher than those fed on cultivar Arg (fig. 4), suggesting the hyperproduction of digestive amylases from midgut cells of *S. cerealella*. Correlation coefficient given in table 3 between amylolytic activity of larvae and inhibition of  $\alpha$ -amylase supported this finding.

According to the correlation analysis, there is a negative correlation between the duration of immature stages of *S. cerealella* and the starch content of tested cultivars (table 3). Behmer (2009) reported that herbivorous insects regulate their nutrient intake using pre- and post-ingestive mechanisms. Since the significant correlation was not established between the amylolytic activity of *S. cerealella* larvae and the starch content of the tested cultivars, it seems that the larvae consumed more food until the requirement for carbohydrate was reached. More feeding by *S. cerealella* larvae may lead to the intake of a high level of enzyme inhibitors into the digestive system that could affect the life history of this pest. As seen in table 3, a significant positive correlation was detected between the duration of immature stages and inhibition of  $\alpha$ -amylase.

### Concluding remarks

The results of this study showed that *S. cerealella* not only tried to complete its life cycle on nutritionally unsuitable wheat cultivars but also developed its immature stages to achieve necessary nutrition for further survival. Moreover, wheat proteinaceous extract, acting as an inhibitor, affected nutrient regulation by *S. cerealella*. The larvae of *S. cerealella* fed on heavier wheat (cultivar Bam) completed their immature stages faster than those reared on lighter cultivars. The results of this study showed that the insects fed on cultivar Sepahan (with the lightest weight) had the longest immature stages, the highest mortality and lowest weights of adult males and females, fecundity and fertility along with a lower amylolytic activity. Therefore, it can be suggested that cultivar Sepahan is an unsuitable host for *S. cerealella*, which could be recommended for cultivation as the most resistant for this pest. An explained biochemical and molecular analysis of midgut

$\alpha$ -amylase enzyme upon disposal of *S. cerealella* to a specific diet will highlight their particular roles. It would be interesting to focus on the digestive proteases of *S. cerealella* and their interactions with the protein content of different wheat cultivars. Also, the insect's response to the  $\alpha$ -amylase inhibitors should be essentially reviewed for the selection of appropriate  $\alpha$ -amylase inhibitors and their transgenic expression for resistance to this pest.

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### References

- Ahmed, S. & Raza, A. (2010) Role of physical properties of maize grains towards resistance to *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera) in no choice test. *Pakistan Entomologist* **32**, 37–42.
- Ahmed, S., Ul-Hasan, M. & Hussain, S. (2002) Studies on the relative susceptibility of wheat varieties to *Sitotroga cerealella* (Oliv.). *Pakistan Journal of Agricultural Sciences* **39**, 47–49.
- A.O.A.C. (1984) *Official Methods of Analysis*. 14th edn. Washington, DC, Association of Official Analytical Chemists.
- Ashamo, M.O. (2010) Relative resistance of paddy varieties to *Sitotroga cerealella* (Lepidoptera: Gelechiidae). *Biologia* **65**(2), 333–337.
- Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* **47**, 817–844.
- Babic, B., Poisson, A., Darwish, S., Lacasse, J., Merckx-Jacques, M., Despland, E. & Bede, J.C. (2008) Influence of dietary nutritional composition on caterpillar salivary enzyme activity. *Journal of Insect Physiology* **54**, 286–296.
- Baker, J.E. (1987) Purification of isoamylases from the rice weevil, *Sitophilus oryzae* L. by HPLC and their interaction with partially purified amylase inhibitor from wheat. *Insect Biochemistry* **17**, 37–44.
- Behmer, S.T. (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology* **54**, 165–187.
- Bentancourt, C.M., Scatoni, I.B., Gonzalez, A. & Franco, J. (2003) Effects of larval diet on the development and reproduction of *Argyrotaenia sphaleropa* (Meyrick) (Lepidoptera: Tortricidae). *Neotropical Entomology* **32**(4), 551–557.
- Bernfeld, P. (1955) Amylases,  $\alpha$  and  $\beta$ . *Methods in Enzymology* **1**, 149–158.
- Bong, C.F.J., Er, C.C., Yiu, P.H. & Rajan, A. (2008) Growth performance of the red-stripe weevil *Rhynchophorus schach* Oliv. (Insecta: Coleoptera: Curculionidae) on meridic diets. *American Journal of Agricultural and Biological Sciences* **3**(1), 403–409.
- Borzoui, E. & Bandani, A.R. (2013) Wheat and triticale proteinaceous seed extracts inhibit gut  $\alpha$ -amylase and protease of the carob moth, *Ectomyelois ceratoniae*. *Molecular Entomology* **4**(3), 13–21.
- Borzoui, E., Naseri, B. & Namin, F.R. (2015) Different diets affecting biology and digestive physiology of the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Journal of Stored Products Research* **62**, 1–7.
- Bouayad, N., Rharrabe, K., Ghailani, N. & Sayah, F. (2008) Effects of different food commodities on larval development and  $\alpha$ -amylase activity of *Plodia interpunctella* (Hubner)



- (Lepidoptera: Pyralidae). *Journal of Stored Products Research* **44**, 373–378.
- Bown, D.P., Wilkinson, H.S. & Gatehouse, J.A.** (1997) Differentially regulated inhibitor-sensitive and insensitive protease genes from the phytophagous insect pest, *Helicoverpa armigera*, are members of complex multigene families. *Insect Biochemistry and Molecular Biology* **27**, 625–638.
- Bradford, M.M.** (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.
- Cinco-Moroyoqui, F.J., Rosas-Burgos, E.C., Barboa-Flores, J. & Cortez-Rocha, M.O.** (2006) Alpha-amylase activity of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) reared on several wheat varieties and its inhibition with kernel extracts. *Journal of Economic Entomology* **99**, 2146–2150.
- Consoli, F.L. & Filho, B.F.A.** (1995) Biology of *Sitotroga cerealella* (Oliv.) (Lepidoptera: Gelechiidae) reared on five corn (maize) genotypes. *Journal of Stored Products Research* **31**(2), 139–143.
- Dastranj, M., Bandani, A.R. & Mehrabadi, M.** (2013) Age-specific digestion of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and inhibition of proteolytic and amylolytic activity by plant proteinaceous seed extracts. *Journal of Asia-Pacific Entomology* **16**, 309–315.
- Dunse, K.M., Kaas, Q., Guarino, R.F., Barton, P.A., Craik, D.J. & Anderson, M.A.** (2010) Molecular basis for the resistance of an insect chymotrypsin to a potato type II proteinase inhibitor. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 15016–15021.
- El Atta, H.A.** (2000) Effect of diet and seed pretreatment on the biology of *Bruchidius uberatus* (Coleoptera, Bruchidae). *Silva Fennica* **34**(4), 431–435.
- Ellington, G.W.** (1930) A method for securing eggs of the Angoumois grain moth. *Journal of Economic Perspectives* **23**, 237–238.
- Fouad, H.A., Faroni, L.R.D., de Lima, E.R. & Vilela, E.F.** (2013) Relationship between physical-chemical characteristics of corn kernels and susceptibility to *Sitotroga cerealella*. *Maydica* **58**, 169–172.
- Franco, O.L., Rigden, D.J., Melo, F.L., Bloch, C. Jr., Silva, C.P. & Grossi de Sa, M.F.** (2000) Activity of wheat  $\alpha$ -amylase inhibitors towards bruchid  $\alpha$ -amylases and structural explanation of observed specificities. *European Journal of Biochemistry* **267**, 2166–2173.
- Gatehouse, A.M.R. & Boulter, D.** (1983) Assessment of the anti-metabolic effects of trypsin inhibitors from cowpea (*Vigna unguiculata*) and other legumes on development of the bruchid beetle *Callosobruchus maculatus*. *Journal of the Science of Food and Agriculture* **34**, 345–350.
- Giri, A.P., Harsulkar, A.M., Deshpande, V.V., Sainani, M.N., Gupta, V.S. & Ranjekar, P.K.** (1998) Chickpea defensive proteinase inhibitors can be inactivated by podborer gut proteinases. *Plant Physiology* **116**, 393–401.
- Hagstrum, D.W. & Subramanyam, B.** (1996) *Integrated Management of Insects in Stored Products*. New York, Marcel Dekker, Inc.
- Hanks, L.M., McElfresh, J.S., Millar, J.G. & Paine, T.D.** (1993) *Phoracantha semipunctata* (Coleoptera, Cerambycidae), a serious pest of *Eucalyptus* in California: biology and laboratory-rearing procedures. *Annals of the Entomological Society of America* **86**(1), 96–102.
- Harsulkar, A.M., Giri, A.P., Patankar, A.G., Gupta, V.S., Sainani, M.N., Ranjekar, P.K. & Deshpande, V.V.** (1999) Successive use of non-host plant proteinase inhibitors required for effective inhibition of *Helicoverpa armigera* gut proteinases and larval growth. *Plant Physiology* **121**, 497–506.
- Hashem, M.Y., Risha, E.S.M., El-Sherif, S.I. & Ahmed, S.S.** (2012) The effect of modified atmospheres, an alternative to methyl bromide, on the susceptibility of immature stages of angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). *Journal of Stored Products Research* **50**, 57–61.
- Hosseininaveh, V., Bandani, A.R., Azmayeshfard, P., Hosseinkhani, S. & Kazzazi, M.** (2007) Digestive proteolytic and amylolytic activities in *Trogoderma granarium* Everts (Dermestidae: Coleoptera). *Journal of Stored Products Research* **43**, 515–522.
- Hosseinkhani, S. & Nemat-Gorgani, M.** (2003) Partial unfolding of carbonic anhydrase provides a method for its immobilization on hydrophobic adsorbents and protects it against irreversible thermoinactivation. *Enzyme and Microbial Technology* **33**, 179–184.
- Jayas, D.S. & Jeyamkondan, S.** (2002) Modified atmosphere storage of grains, meat, fruits and vegetables. *Biosystems Engineering* **82**, 235–251.
- Karasov, W.H., Martinez del Rio, C. & Caviedes-Vidal, E.** (2011) Ecological physiology of diet and digestive systems. *Annual Review of Physiology* **73**, 69–93.
- Kazzazi, M., Bandani, A.R. & Hosseinkhani, S.** (2005) Biochemical characterization of  $\alpha$ -amylase of Sunn pest *Eurygaster integriceps*. *Entomological Science* **8**, 371–377.
- Khan, I., Afsheen, S., Din, N., Khattak, S.U.K., Khalil, S.K., Hayat, Y. & Lou, Y.** (2010) Appraisal of different wheat genotypes against Angoumois grain moth, *Sitotroga cerealella* (Oliv.). *Pakistan Journal of Zoology* **42**(2), 161–168.
- Khattak, S.U., Munaf, A., Khalil, S.K., Hussain, A. & Hussain, N.** (1996) Relative susceptibility of wheat cultivars to *Sitotroga cerealella* (Oliv.). *Pakistan Journal of Zoology* **28**(2), 115–118.
- Kotkar, H.M., Sarate, P.J., Tamhane, V.A., Gupta, V.S. & Giri, A.P.** (2009) Responses of midgut amylases of *Helicoverpa armigera* to feeding on various host plants. *Journal of Insect Physiology* **55**, 663–670.
- Lomate, P.R. & Hivrale, V.K.** (2011) Differential responses of midgut soluble aminopeptidases of *Helicoverpa armigera* to feeding on various host and non-host plant diets. *Arthropod-Plant Interactions* **5**, 359–368.
- Melo, F.R., Sales, M.P., Silva, L.S., Franco, O.L., Bloch, J.R.C. & Ary, M.B.** (1999)  $\alpha$ -Amylase inhibitors from cowpea seeds. *Protein and Peptide Letter* **6**, 387–392.
- Mendiola-Olaya, E., Valencia-Jimenez, A., Valdes-Rodriguez, S., Delano-Frier, J. & Blanco-Labra, A.** (2000) Digestive amylase from the larger grain borer, *Prostephanus truncatus* Horn. *Comparative Biochemistry and Physiology B* **126**, 425–433.
- Nay, J.E. & Perring, T.M.** (2008) Influence of host plant stages on carob moth (Lepidoptera: Pyralidae) development and fitness. *Environmental Entomology* **37**(2), 568–574.
- Ohmart, C.P.** (1991) Role of food quality in the population dynamics of chrysomelid beetles feeding on *Eucalyptus*. pp. 35–46, vol. 39 in Raske, A.G. & Wickman, B.E. (Eds) *Proceedings of a Symposium, Towards Integrated Pest Management of Forest Defoliators, Held at the 18th International Congress of Entomology*. Vancouver, Canada, Forest Ecology and Management.
- Pashley, D.P., Ardí, T.N. & Hammond, A.M.** (1995) Host effects on developmental and reproductive traits in fall armyworm strains (Lepidoptera: Noctuidae). *Annals of the New York Academy of Sciences* **88**, 748–755.

- Pytelkova, J., Hubert, J., Lepsik, M., Sobotnik, J., Sindelka, R., Krizkova, I., Horn, M. & Mares, M. (2009) Digestive  $\alpha$ -amylases of the flour moth *Ephestia kuehniella*-adaptation to alkaline environment and plant inhibitors. *FEBS Journal* **276**, 3531–3546.
- Saikia, J., Goswami, M.M. & Bhattacharyya, B. (2014) Biology and detection technique of Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) on stored rice and maize grains. *Journal of Entomology and Zoology Studies* **2** (6), 9–11.
- Sarate, P.J., Tamhane, V.A., Kotkar, H.M., Ratnakaran, N., Susan, N., Gupta, V.S. & Giri, A.P. (2012) Developmental and digestive flexibilities in the midgut of a polyphagous pest, the cotton bollworm, *Helicoverpa armigera*. *Journal of Insect Science* **12**, 42. Available online at <http://insectscience.org/12.42>
- SAS (2003) *A Guide to Statistical and Data Analysis*. Version 9.1. Cary, SAS Institute.
- Shafique, M., Ahmad, M. & Chaudry, M.A. (2006) Evaluation of wheat varieties for resistance to Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). *Pakistan Journal of Zoology* **38**(1), 7–10.
- Shazali, M.E.H. (1987) Weight loss caused by development of *Sitophilus oryzae* (L.) and *Sitotroga cerealella* (Oliv.) in sorghum grains of two size classes. *Journal of Stored Products Research* **23** (4), 233–238.
- Shobana, K., Murugan, K. & Kumar, A.N. (2010) Influence of host plants on feeding, growth and reproduction of *Papilio polytes* (the common mormon). *Journal of Insect Physiology* **56**, 1065–1070.
- Shukle, R.H. & Wu, L. (2003) The role of protease inhibitors and parasitoids on the population dynamics of *Sitotroga cerealella* (Lepidoptera: Gelechiidae). *Environmental Entomology* **32**(3), 488–498.
- Silva, C.P., Terra, W.R., Xavier-Filho, J., Grossi de Sa, M.F., Isejima, E.M., DaMatta, R.A., Miguens, F.C. & Bifano, T.D. (2001) Digestion of legume starch granules by larvae of *Zabrotes subfasciatus* (Coleoptera: Bruchidae) and the induction of  $\alpha$ -amylases in response to different diets. *Insect Biochemistry and Molecular Biology* **31**, 41–50.
- Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T. & Raubenheimer, D. (2004) Optimal foraging when regulating intake of multiple nutrients. *Animal Behavior* **68**, 1299–1311.
- Tabatabaei, P.R., Hosseinaveh, V., Goldansaz, S.H. & Talebi, K. (2011) Biochemical characterization of digestive proteases and carbohydrases of the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology* **14**, 187–194.
- Terashima, M. & Katoh, S. (1996) Modification of  $\alpha$ -amylase function by protein engineering. *Annals of the New York Academy of Sciences* **799**, 65–69.
- Throne, J.E. & Weaver, D.K. (2013) Impact of temperature and relative humidity on life history parameters of adult *Sitotroga cerealella* (Lepidoptera: Gelechiidae). *Journal of Stored Products Research* **55**, 128–133.
- Weston, P.A. & Rattlingourd, P.L. (1999) Ovipositional stimuli of Angoumois grain moth (Lepidoptera: Gelechiidae), a primary pest of stored grains. *Journal of Entomological Science* **34**, 445–451.