

# Geographic Origin and Host Cultivar Influence on Digestive Physiology of *Spodoptera exigua* (Lepidoptera: Noctuidae) Larvae

Neshat Golikhajeh,<sup>1</sup> Bahram Naseri,<sup>1,2</sup> and Jabraeil Razmjou<sup>1</sup>

<sup>1</sup>Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran, and <sup>2</sup>Corresponding author e-mail: bnaseri@uma.ac.ir

Subject Editor: Bill Bendena

Received 24 August 2016; Editorial decision 1 November 2016

## Abstract

Digestive enzymatic activity in three geographic strains (Miandiab, Kalposh and Moghan regions) of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) reared on different sugar beet cultivars (Dorothea, Rozier, Persia and Perimer) was studied under laboratory conditions ( $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and a photo period of 16:8 (L:D) h photoperiod). The results of this study demonstrated that digestive protease and amylase activity of *S. exigua* larvae was affected by both geographic origin of the pest and host plant cultivar. Three strains reared on the same sugar beet cultivars demonstrated different levels of proteolytic and amylolytic activities in fourth and fifth instars. The highest proteolytic and amylolytic activity, in most cases, was observed in larvae collected from Kalposh region. Among different sugar beet cultivars, the highest protease activity in three strains was observed on cultivars Rozier and Perimer. Nevertheless, the highest amylase activity was seen on cultivar Dorothea, and the lowest activity was seen on cultivar Rozier. This study suggested that variations in digestive enzymatic activity of three geographic strains of *S. exigua* might be attributed to local adaptation with their local host plant and environmental conditions inherent by larvae.

**Key words:** sugar beet cultivar, geographic population, enzymatic activity, local host plant

Sugar beet, *Beta vulgaris* (L.) (Chenopodiaceae) is an important agricultural crop, grown commercially for sugar production in various countries around the world including Iran (Shah-Smith and Burns 1997, Collins and Jacobsen 2003, Biancardi et al. 2012). Among sugar beet pests, *Spodoptera exigua* (Hübner) is introduced as the most harmful species that attacks all growth steps of this plant (Saghfi and Valizadegan 2014). *S. exigua* is a polyphagous species that can feed on over 50 species from over 10 plant families worldwide (Smits et al. 1987). Due to the high migration capacity of *S. exigua* in long distances (Mitchell 1979), study of geographic strains of this pest is one of the important factors for analyzing its populations (Adamczyk et al. 2003). Among different biotic and abiotic factors influencing the life cycle of *S. exigua*, host plants and climate conditions could greatly affect the development of this pest (Chen et al. 2008, Karimi-Malati et al. 2014), and play a significant role in the regulation of Lepidoptera populations (Singh and Parihar 1988, Lu and Xu 1998).

Due to the importance of sugar beet as a source of sucrose, and the resistance of *S. exigua* to various insecticides, alternative control methods are needed to prevent the economic damages caused by this insect. One of the alternative techniques in integrated pest management programs is the study of the insects' digestive physiology

(Lawrence and Koundal 2002). The activity of the insects' digestive enzymes can be affected by several factors like the amount and quality of food diet, temperature, and gut acidity (Sivakumar et al. 2006). The secretion of midgut digestive enzymes such as proteases, amylases, and lipases catalyzes the digestion of food macromolecules (Pauchet et al. 2008). Digestive protease is a midgut and salivary enzyme that catalyzes the release of peptides and amino acids from proteins in an insect digestive system. Furthermore, amylase is involved in hydrolysis of starch and other carbohydrates, and activity of this enzyme depends on food diet (Terra and Ferreira 1994).

Plant species and diversity in regional zones (Davidson et al. 2001), chemical composition of host plant (Foss and Rieske 2003), and age of plant are important factors involved in host plant preference by insect pests (Meyer and Montgomery 2004). Herbivorous insects can overcome the harmful effects of defensive compounds of host plants employing different mechanisms like digestive and detoxification enzymes (Mello and Silva-Filho 2002, Zhu-Salzman et al. 2005).

Due to the economic damages caused by different strains of *S. exigua* to numerous crops, and its resistance to synthetic insecticides (Brewer and Trumble 1989, Layton 1994, Chi et al. 2013), many scientists have studied host plant effects on the growth and life

history (Greenberg et al. 2001; Showler and Moran 2003; Telang et al. 2001; Lee et al. 2002; Farahani et al. 2011), nutritional responses (Mehrkhou 2013, Mehrkhou et al. 2015) as well as digestive enzymatic activity of *S. exigua* (Wang et al. 2003; Wu and Wang 2003; Li et al. 2005; Zhang et al. 2011a,b). Nevertheless, no studies have been published regarding the effect of geographic origin of *S. exigua* on its digestive physiology. Thus in this research, the digestive physiology of three geographic strains of larvae when reared on four different sugar beet cultivars was studied. It was expected to observe variations in the digestive enzymatic activity (protease and amylase) of *S. exigua* collected from different regions. The results of this study may well be useful in identifying improved techniques for managing this pest and understanding the plant-insect interactions in any particular region.

## Materials and Methods

### Sugar Beet Sources

Seeds of four tested sugar beet (*B. vulgaris*) cultivars including Dorothea, Persia, Rozier, and Perimer were obtained from the Plant and Seed Modification Research Institute of Sugar Beet (Ardabil, Iran). Selected cultivars were grown in the research farm of the University of Mohaghegh Ardabili (Ardabil, Iran) in May 2014. These cultivars are the most cultivated sugar beets in different regions of Iran.

### Collection and Rearing of *S. exigua*

Larval strains of *S. exigua* were collected from sugar beet farms from three regions, which had the highest production of sugar beet in Iran; including Semnan (Kalposh) collected from cultivar Perimer, Western Azerbaijan (Miandoab) collected from cultivar Dorothea and Ardabil (Moghan) collected from cultivar Rozier. To remove the effect of prior feeding experience on local host plant as well as providing similar rearing conditions for different strains, individuals from each region were kept separately and reared for two generations on another sugar beet cultivar named Torbat. Thirty newly hatched larvae of each strain of *S. exigua* from third generation were transferred into plastic containers (diameter 16.5 cm, depth 7.5 cm), containing fresh leaves of each tested cultivar (in four leaf stage). The outlets of these containers were covered by a mesh net for larval aeration. To maintain the freshness of the sugar beet leaves, the petioles of detached leaves were inserted in water-soaked cotton. All tested insects were reared in a growth chamber at  $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH, and a photoperiod of 16:8 (L:D) h.

### Chemicals

The enzyme substrates (starch and azocasein), the Bradford reagent, and the dinitrosalicylic acid were obtained from Sigma Chemical Co. (Sigma-Aldrich, www.sigmaaldrich.com). Bovine serum albumin (BSA), potassium iodine (KI), and acetic acid were purchased from Merck Co. (Merck, www.merck.com). Iodine (I<sub>2</sub>) was obtained from Maarsen Co.

### Preparation of Digestive Enzymes

The fourth and fifth instars of each geographic strain of *S. exigua* were fed (for 24 h) with leaves of four tested sugar beet cultivars, and immediately dissected under a stereoscopic microscope. Five midguts of fourth and fifth instars were washed in cold distilled water and were submerged in 1.5 ml distilled water. The homogenates were centrifuged at  $16,000 \times g$  at  $4^\circ\text{C}$  for 10 min and the resulting

supernatants were collected in new micro tubes and stored at  $-20^\circ\text{C}$  in aliquots for further use.

### Protein Concentration of Larvae

General protein concentrations in the midguts of fourth and fifth instars from each strain of *S. exigua* fed with tested sugar beet cultivars were determined using BSA as a standard based on the procedure described by Bradford (1976).

### Proteolytic Activity Assay

General protease activity in larval midgut of three geographic strains of *S. exigua* fed with tested sugar beet cultivars (for 24 h) was assayed utilizing azocasein (1.5%) substrate at optimal pH 12 (Elpidina et al. 2001).

### Amylolytic Activity Assay

The  $\alpha$ -amylase activity in larval midgut of three geographic strains of *S. exigua* fed with four tested sugar beet cultivars was measured employing the method of Bernfeld (1955), with 1% soluble starch as substrate.

### Protein, Starch, and Proline Contents in Leaves of Sugar Beet Cultivars

Protein content of the sugar beet cultivars was quantified through BSA as standard according to Bradford (1976). A quantity of 200 mg of each sugar beet cultivar leaf was homogenized in 10 ml of distilled water. One hundred microliters of the homogenate were thereafter added to 3 ml of Bradford reagent. The samples were incubated in darkness at  $37^\circ\text{C}$  and the absorbance was read at 595 nm.

Starch content of tested sugar beet cultivars was determined by the method of Bernfeld (1955) utilizing starch as standard. A quantity of 200 mg of each sugar beet cultivar leaf was homogenized in 35 ml of distilled water and heated to boiling point. In total, 100 ml of each sample were 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.

Proline content in the leaves of sugar beet cultivars was determined according to the method described by Bates et al. (1973).

### Data Analysis

The assay of digestive enzymatic activity of *S. exigua* was performed by factorial design with two main factors (strain in three levels and cultivar in four levels) and was analyzed with two-way ANOVA utilizing SAS program (PROC GLM, SAS Institute 1989). Data for larval protein content, protein, starch, and proline concentrations in each cultivar were analyzed with one-way ANOVA using SAS program (PROC GLM, SAS Institute 1989). The means were compared with LSD test at  $\alpha = 0.05$ . All data were tested for normality before analysis by Kolmogorov-Smirnov test, which were normally distributed.

## Results

Digestive enzymatic activity in three geographic strains of *S. exigua* in response to feeding on four tested sugar beet cultivars was studied in fourth and fifth instars. In this study, through the use of factorial design, the effects of *S. exigua* strain, sugar beet cultivar and their interaction were studied on digestive enzymatic activity and larval protein content of this pest (Table 1). To summarize the comparison of digestive enzymatic activity among geographic strains of *S. exigua* fed on different sugar beet cultivars, only the interaction effects (strain  $\times$  cultivar) are given here.

**Table 1.** Statistics of analysis of variance for the effect of strain, sugar beet cultivar, and their interaction on enzymatic (proteolytic and amylolytic) activity and midgut protein content of fourth and fifth larval instar of *S. exigua*

| Enzyme activity      | Source of variation | Degrees of freedom | Fourth instar |        | Fifth instar |        |
|----------------------|---------------------|--------------------|---------------|--------|--------------|--------|
|                      |                     |                    | F             | P      | F            | P      |
| Proteolytic activity | Strain              | 2                  | 5.64          | 0.0098 | 10.27        | 0.0006 |
|                      | Cultivar            | 3                  | 28.15         | 0.0001 | 21.81        | 0.0001 |
|                      | Strain × cultivar   | 6                  | 7.55          | 0.0001 | 9.44         | 0.0001 |
|                      | Error               | 24                 |               |        |              |        |
| Amylolytic activity  | Strain              | 2                  | 23.61         | 0.0001 | 20.94        | 0.0001 |
|                      | Cultivar            | 3                  | 7.54          | 0.0010 | 25.04        | 0.0001 |
|                      | Strain × cultivar   | 6                  | 10.61         | 0.0001 | 27.95        | 0.0001 |
|                      | Error               | 24                 |               |        |              |        |
| Protein content      | Strain              | 2                  | 25.05         | 0.0001 | 12.77        | 0.0002 |
|                      | Cultivar            | 3                  | 15.20         | 0.0001 | 15.46        | 0.0001 |
|                      | Strain × cultivar   | 6                  | 14.07         | 0.0001 | 2.57         | 0.0455 |
|                      | Error               | 24                 |               |        |              |        |

### Effect of *S. exigua* Strain and Sugar Beet Cultivar on Protease Activity

The effect of geographic strain and sugar beet cultivar on protease activity of fourth and fifth instars of *S. exigua* is shown in Figure 1. Among strains, Miandoab strain of fifth instar demonstrated the highest protease activity on cultivar Rozier ( $F = 6.49$ ;  $df = 2, 6$ ;  $P = 0.032$ ), whereas; Kalposh strain had the highest protease activity on cultivars Perimer ( $F = 18.87$ ;  $df = 2, 6$ ;  $P = 0.003$ ) and Dorothea ( $F = 36.34$ ;  $df = 2, 6$ ;  $P = 0.0001$ ). Within strains, fourth instar of Miandoab reared on cultivar Persia ( $F = 6.63$ ;  $df = 3, 8$ ;  $P = 0.015$ ) and Kalposh reared on cultivars Dorothea and Rozier ( $F = 48.29$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) had the highest protease activity. Fifth instar of Miandoab ( $F = 19.30$ ;  $df = 3, 8$ ;  $P = 0.001$ ) and Moghan ( $F = 51.32$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) strains reared on cultivar Rozier demonstrated the highest protease activity.

### Effect of *S. exigua* Strain and Sugar Beet Cultivar on Amylase Activity

Figure 2 shows the effect of geographic strain and sugar beet cultivar on amylase activity of fourth and fifth instars of *S. exigua*. Among strains, the highest amylase activity of fourth instar was seen for Miandoab strain reared on cultivars Persia ( $F = 16.66$ ;  $df = 2, 6$ ;  $P = 0.004$ ) and Perimer ( $F = 161.45$ ;  $df = 2, 6$ ;  $P = 0.0001$ ), and for Kalposh strain reared on cultivar Dorothea ( $F = 49.64$ ;  $df = 2, 6$ ;  $P = 0.0001$ ). In the fifth instar, the highest amylase activity was detected in Kalposh strain reared on cultivar Dorothea ( $F = 56.65$ ;  $df = 2, 6$ ;  $P = 0.0001$ ), and Miandoab strain reared on cultivar Perimer ( $F = 19.18$ ;  $df = 2, 6$ ;  $P = 0.002$ ).

Within each strain, the highest and lowest amylase activity of the fourth instar of Miandoab strain ( $F = 24.79$ ;  $df = 3, 8$ ;  $P = 0.045$ ) was observed on cultivars Persia and Rozier, respectively. Moghan strain ( $F = 9.25$ ;  $df = 3, 8$ ;  $P = 0.006$ ) reared on cultivar Dorothea demonstrated the lowest amylase activity. Kalposh strain ( $F = 4.52$ ;  $df = 3, 8$ ;  $P = 0.039$ ) reared on cultivars Dorothea and Perimer demonstrated the highest and lowest amylase activity, respectively. The highest and lowest amylase activity of fifth instar of Miandoab strain ( $F = 21.60$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) was observed on cultivars Perimer and Dorothea, respectively. Kalposh ( $F = 19.58$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) strain reared on cultivar Dorothea demonstrated the highest amylase activity.

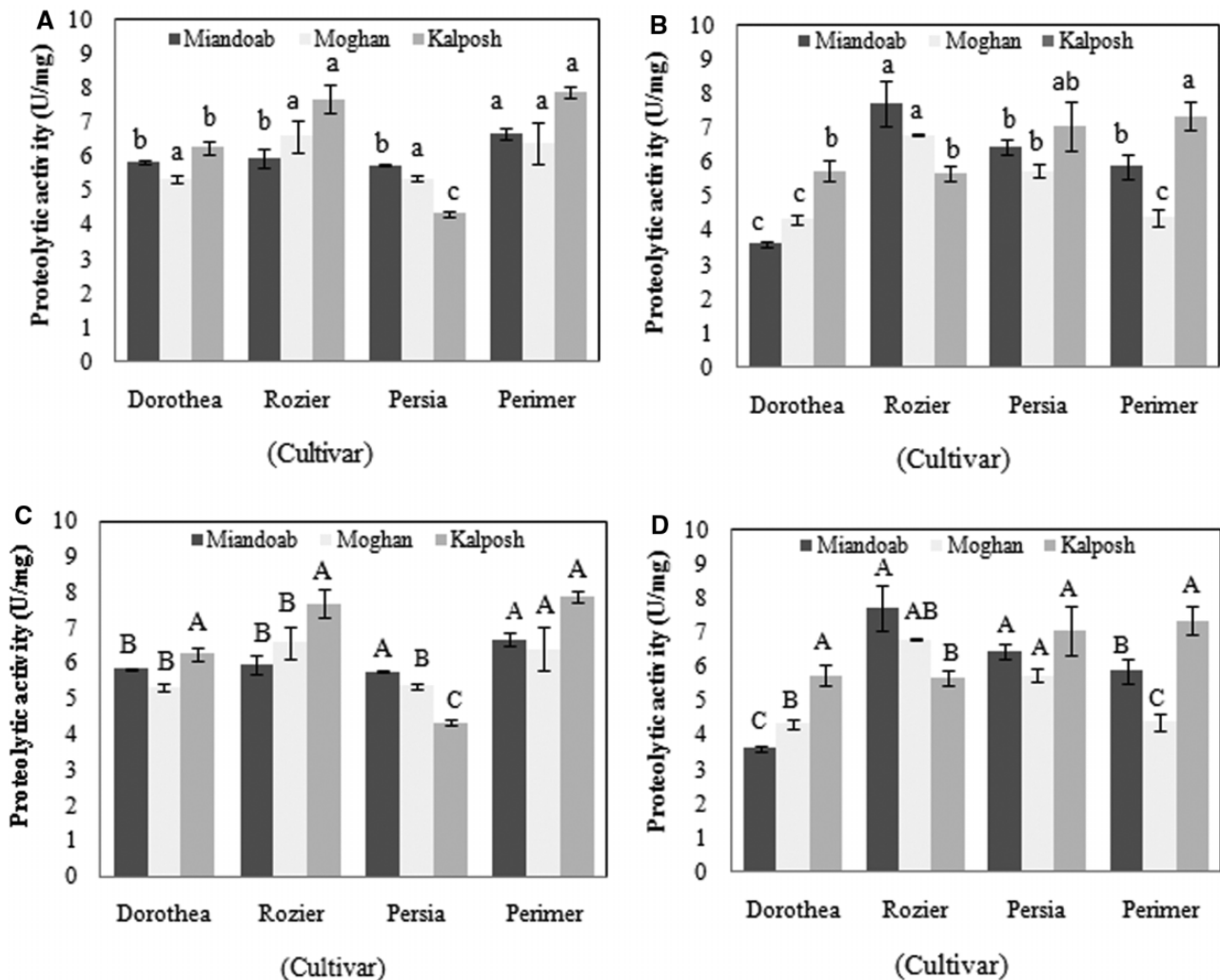
### Effect of *S. exigua* Strain and Sugar Beet Cultivar on Larval Protein Content

Effect of *S. exigua* strain and sugar beet cultivar on midgut protein content of fourth and fifth instars of *S. exigua* is presented in Figure 3. In the fourth instar, the highest larval protein content of Miandoab strain was observed on cultivars Perimer ( $F = 45.93$ ;  $df = 2, 6$ ;  $P = 0.0001$ ) and Rozier ( $F = 26.68$ ;  $df = 2, 6$ ;  $P = 0.001$ ). The Moghan strain reared on cultivar Dorothea ( $F = 11.63$ ;  $df = 2, 6$ ;  $P = 0.009$ ) and Kalposh strain reared on cultivar Persia ( $F = 71.03$ ;  $df = 2, 6$ ;  $P = 0.0001$ ) showed the highest protein content. In the fifth instar, Moghan strain reared on cultivar Perimer ( $F = 55.26$ ;  $df = 2, 6$ ;  $P = 0.0001$ ) showed the highest protein content while Kalposh strain reared on cultivar Persia ( $F = 36.43$ ;  $df = 2, 6$ ;  $P = 0.0001$ ) showed the lowest protein content.

Within each strain, Miandoab strain of the fourth instar ( $F = 111.09$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) reared on cultivar Rozier showed the highest protein content. Moghan strain ( $F = 10.32$ ;  $df = 3, 8$ ;  $P = 0.004$ ) reared on cultivar Dorothea exhibited the highest protein content. Kalposh strain ( $F = 36.65$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) reared on cultivars Persia and Rozier exhibited the highest and lowest protein content, respectively. Fifth instar larvae of Miandoab strain ( $F = 105.81$ ;  $df = 3, 8$ ;  $P = 0.0001$ ) fed on cultivars Dorothea and Rozier showed the highest and lowest protein content, respectively. The highest and lowest larval protein contents of Moghan strain ( $F = 2.56$ ;  $df = 3, 8$ ;  $P = 0.012$ ) were seen on cultivars Perimer and Rozier, respectively. In Kalposh strain ( $F = 2.87$ ;  $df = 3, 8$ ;  $P = 0.015$ ), larvae reared on cultivar Rozier exhibited the highest protein content, and those reared on cultivar Persia had the lowest protein content.

### Protein, Starch, and Proline Determination of Tested Sugar Beet Cultivars

Figure 4 shows protein, starch and proline contents in the leaf of four tested sugar beet cultivars. Our data showed significant difference in protein ( $F = 42.44$ ;  $df = 3, 8$ ;  $P = 0.0001$ ), starch ( $F = 41.87$ ;  $df = 3, 8$ ;  $P = 0.001$ ) and proline ( $F = 110.27$ ;  $df = 3, 8$ ;  $P = 0.001$ ) contents of various sugar beet cultivars. The highest and lowest protein contents were recorded in cultivars Persia and Rozier, respectively. Cultivar Dorothea had the highest content of starch; while, cultivars Persia and Perimer had the lowest content of starch. The highest proline content was observed in cultivar Perimer, and the lowest content was seen in cultivars Persia and Rozier.



**Fig. 1.** Effect of geographic origin and sugar beet cultivar on proteolytic activity (mean  $\pm$  SEM) of fourth (A, C) and fifth (B, D) instar of *S. exigua*. The means followed by different lower case letters for each strain reared on four sugar beet cultivars and different upper case letters for each strain reared on the same sugar beet cultivar are significantly different (LSD test).

## Discussion

In this study, protease and  $\alpha$ -amylase activities in larval midgut of three geographic strains of *S. exigua* in response to feeding on four tested sugar beet cultivars were measured. The present data indicate that both geographic strain of *S. exigua* and host plant quality influence the digestive enzymatic activity of this insect.

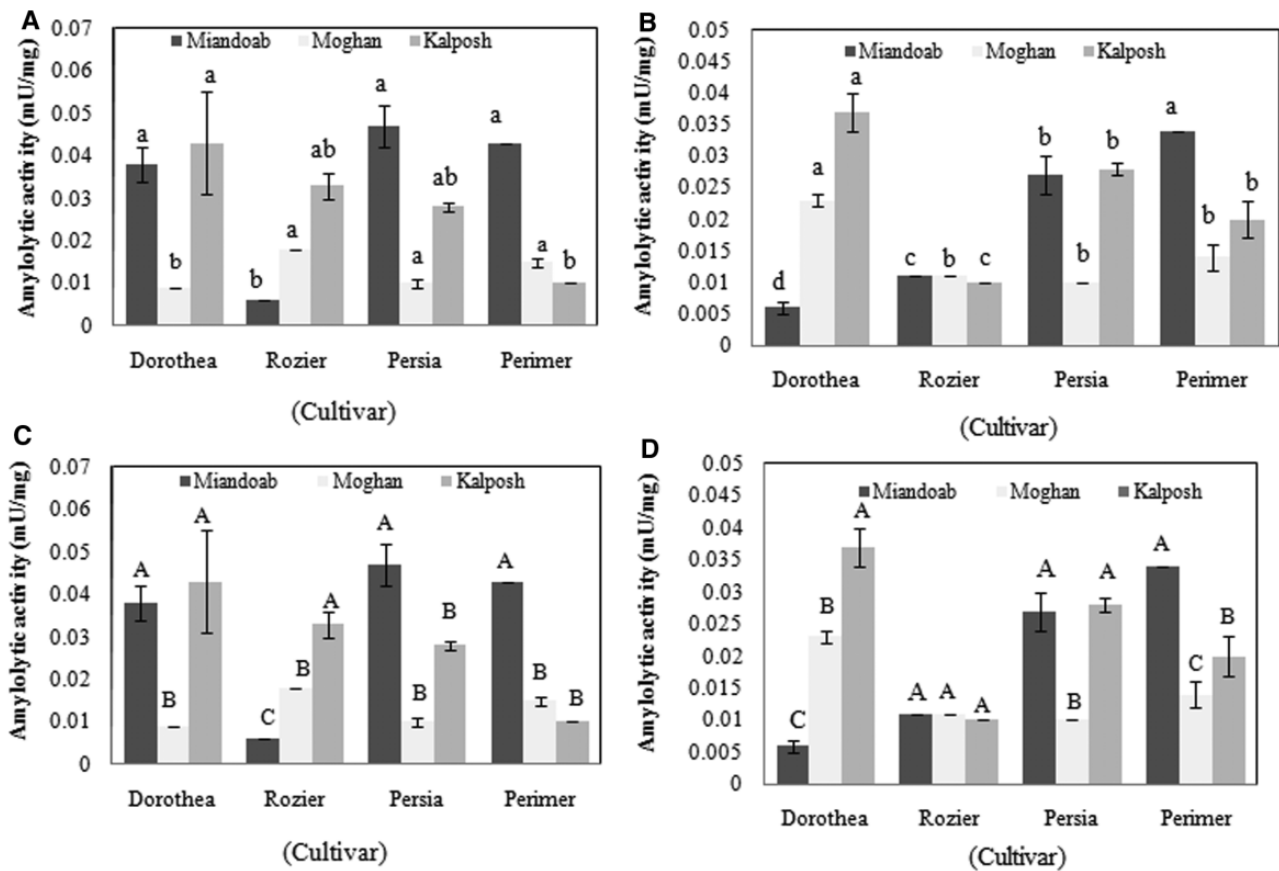
As shown in Figures 1 and 2, lower protease activity in Moghan strain and higher amylase activity in Miandoab strain might be attributed to prior feeding experience of these strains on regional host plants as well as the effect of environmental conditions inherent by strains. Several studies indicated that oviposition preferences for specific host plants (Coyle et al. 2011, Anderson et al. 2013) and variation in behavior of host plant preference by herbivores (Prokopy and Lewis 1993) are inducible by prior experience of larvae or adult with the plant.

Our study indicated that, in most cases, larval strains reared on local host plant (Miandoab strain on cultivar Dorothea, Moghan strain on cultivar Rozier and Kalposh strain on cultivar Perimer) had higher enzymatic activity when compared with larvae reared on non-local host plants. Insect populations generally exhibited greater fitness on local host plants than plants from other regions

(Thompson 2005, Singer and McBride 2009). Local adaptation is an evolutionary process, which facilitates an organism's survival in a particular environment (Williams 1966). As a result of their larger population sizes, shorter generation times, and higher mutation rates (Ebert 1994, Dybdahl and Storfer 2003), the herbivorous insects are expected to exhibit local adaptation in herbivore-host interactions. Rausher (1982) reported that faster development and better survivorship in different populations of *Euphydrya editha* (Lepidoptera: Nymphalidae) reared on local host plant than other non-local host plant may well be as a result of the differences in feeding behavior and digestive physiology of the pest.

Among different sugar beet cultivars in three strains (Fig. 1), the highest protease activity was on cultivar Rozier; however, this cultivar exhibited relatively lower protein content. Perhaps, higher food consumed by the larvae or the presence of some proteinase inhibitors; influence the insect to synthesize more digestive enzymes by the midgut cells. The food consumption and utilization in insects had a direct effect on the activity of digestive enzymes (Sivakumar et al. 2006).

The highest amylase activity on cultivar Dorothea (Fig. 2) might be attributed to higher starch content in this cultivar. It is accepted that the primary nutrients (especially protein and starch contents)



**Fig. 2.** Effect of geographic origin and sugar beet cultivar on amylolytic activity (mean  $\pm$  SEM) of fourth (A, C) and fifth (B, D) instar of *S. exigua*. The means followed by different lower case letters for each strain reared on four sugar beet cultivars and different upper case letters for each strain reared on the same sugar beet cultivar are significantly different (LSD test).

and secondary biochemicals of host plants (Wang et al. 2006) can influence the digestive enzymatic activity of *S. exigua*. As seen in Figure 4, proline content varied between sugar beet cultivars. Proline is one of the key components of a plant defense in response to various stresses. As reported by Khattab and Khattab (2005), proline content increased in eucalyptus leaves attacked by xylem-feeding insects. Proline can protect plants against stress by acting as a storage compound for both carbon and nitrogen sources (Serrano and Gaxiola 1994). Variations among three geographical strains of *S. exigua* on different tested cultivars indicate that nutritional value of these cultivars significantly influenced the protease and amylase activities of this pest.

The insects can change enzymatic activity when they encounter unfavorable conditions that may directly influence their resistance to these conditions (Terriere 1984, Konarev 1996). Vazquez-Arista et al. (1999) reported that variations in digestive enzymatic activity of three colonies of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) from three geographic regions might be attributed to the genetic adaptation of the insect with different environmental conditions. These variations in enzymatic activity, in our study, might be attributed to the adaptation with environmental conditions or their regional host plant, where larvae were collected. Since *S. exigua* is distributed in a wide range, variation in digestive enzymatic activity of different geographic populations is expected as a result of climate differences. Studying the demographic parameters of different geographic populations of *S. exigua*, Golikhajeh et al. (2016) reported that climate factors influenced life cycle and

population growth of *S. exigua*. Temperature can change insects' life history, voltinism, population density, size, genetic composition, extent of host plant exploitation (both in time and space), and geographical distribution (Bale et al. 2002). Data of some climatic factors in spring 2014 and number of frost days in autumn and winter 2013 in the tested geographic regions indicated that Miandoab region had the highest frost days and the lowest mean temperature compared with Kalposh and Moghan regions (Golikhajeh et al. 2016). Atapour and Moharrampour (2014) noted that temperature, especially during fall and winter, plays an important role on the population of *S. exigua*. They reported that the importance of *S. exigua* as main pest of sugar beet in Iran in recent years has reduced as a result of increase in winter temperature. Therefore, lower temperature and longer frost days in Miandoab region could be a main reason for better survival, development and fecundity of *S. exigua* than the other regions. Low winter temperature might be beneficial as a result of reducing winter mortality and enhancing adult fecundity of insects. In contrast, mild winter might be detrimental since more energy is consumed by larvae in the winter (Danks 1987). In the most cases, higher larval protein content and enzymatic activity in Kalposh and Miandoab strains than Moghan strain indicated that larvae collected from these regions had higher energy for host plant use which led to higher speed of food intake to body biomass. Low temperature in winter is beneficial to increase energy conservation in *Eurosta solidaginis* (Diptera: Tephritidae) and other non-feeding winter diapauses species (Tauber et al. 1986) because conservation of post-winter energy reserves can lead to high dispersal and

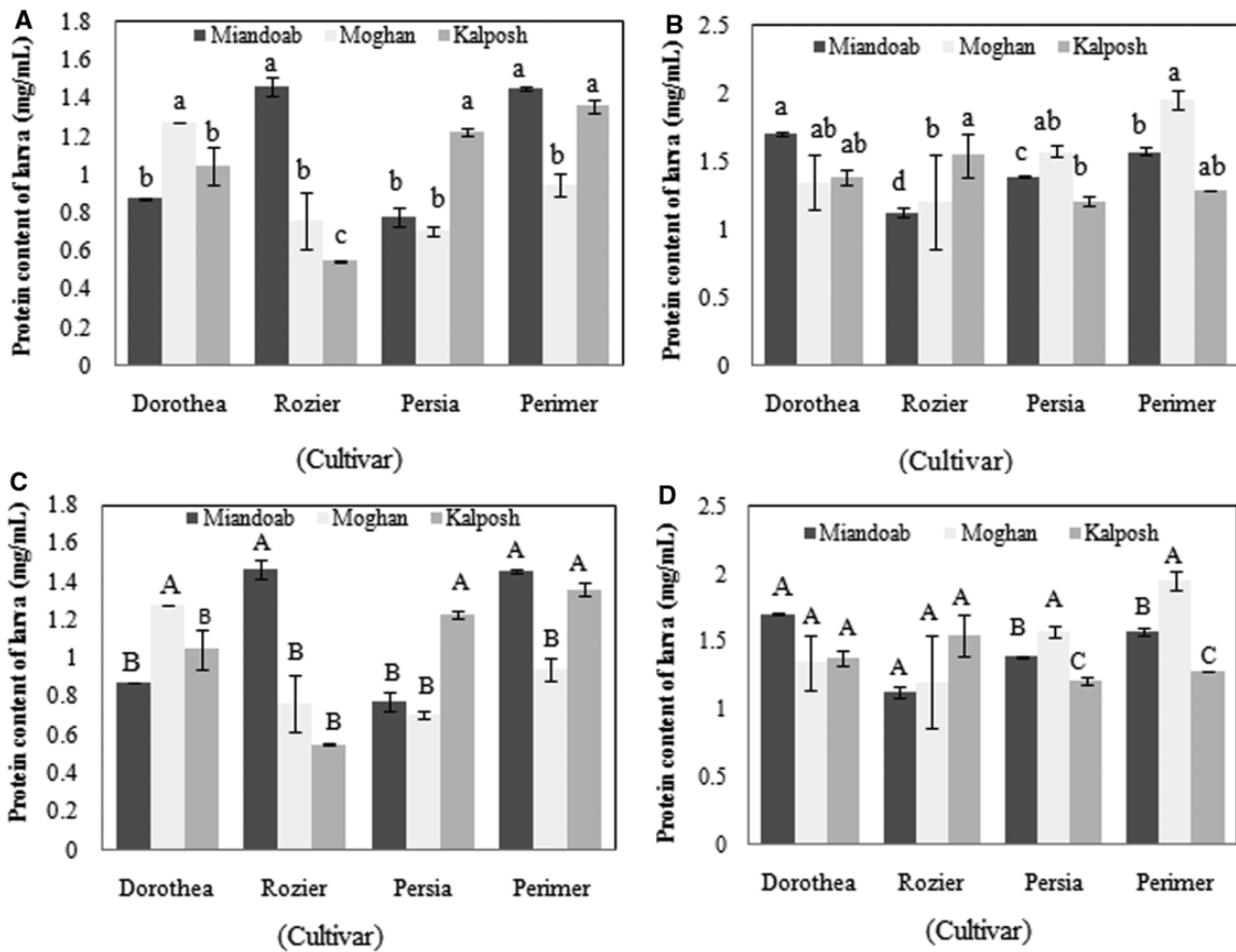


Fig. 3. Effect of geographic origin and sugar beet cultivar on larval protein content (mean  $\pm$  SEM) of fourth (A, C) and fifth (B, D) instar of *S. exigua*. The means followed by different lower case letters for each strain reared on four sugar beet cultivars and different upper case letters for each strain reared on the same sugar beet cultivar are significantly different (LSD test).

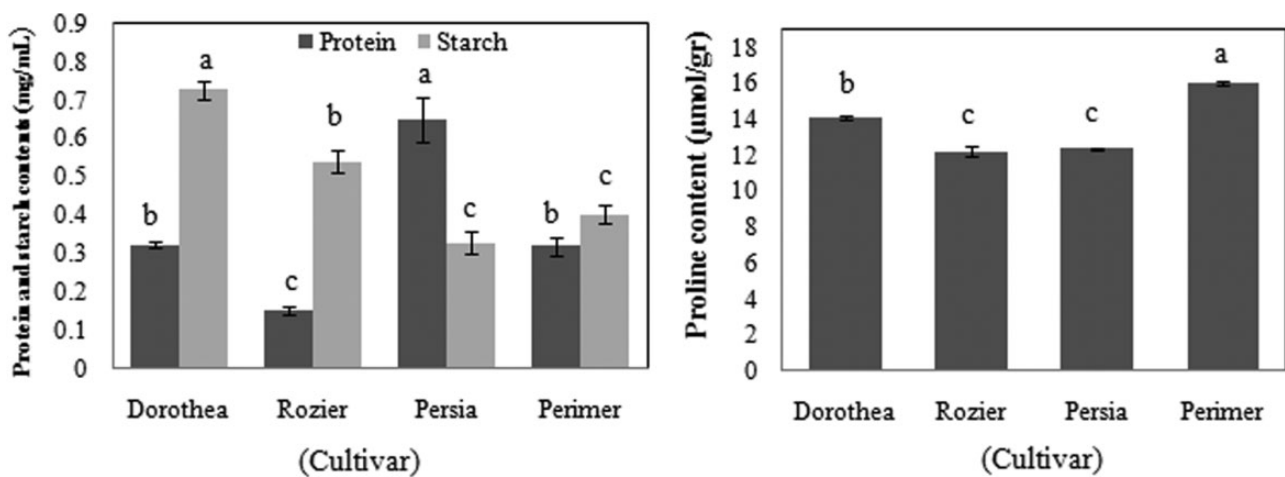


Fig. 4. Mean ( $\pm$ SEM) protein, starch and proline contents of four tested sugar beet cultivars used for feeding of *S. exigua*.

reproductive potential of the pest (Irwin and Lee 2000). Climate changes can directly influence the insect physiology and behavior (Bale et al. 2002, Parmesan 2007, Merrill et al. 2008) or indirectly mediate by host plants, competitors or natural enemies (Harrington

et al. 2001, Bale et al. 2002, Thomson et al. 2010). In this study, no evidence for genetic variations was mentioned among strains, but our previous works on genetic variation of seven populations of *S. exigua* in Iran (unpublished data) demonstrated a high variation

among populations, especially between Kalposh and Moghan populations. So, genetic variation may possibly be one of the important reasons for differences in enzymatic activity of larvae between these strains.

Although it is expected that host plant quality might affect larval performance and activity of the enzymes, this is the first study to demonstrate the effect of geographic origin and prior feeding experience of *S. exigua* on digestive enzymatic activity of this pest.

In conclusion, to obtain more knowledge as regards the geographic distribution effect on the digestive physiology of *S. exigua*, more studies on biological parameters and genetic variation of geographic strains of this pest on local sugar beet cultivars are required. Moreover, understanding the relationship between geographic strain and digestive enzymes of herbivorous insects will make a significant contribution to our knowledge of the highly complex nature of plant-herbivore interactions. This study demonstrated that although sugar beet cultivars influenced digestive enzymatic activity of *S. exigua*, prior feeding experience of the pest on its local host plant and geographic origin of the insect had larger effect than the cultivar of host plant.

## Acknowledgements

We gratefully appreciate M. Ghollarata for her valuable assistance in this research.

## Funding

This work was financially supported by the University of Mohaghegh Ardabili (Ardabil, Iran).

## References Cited

- Adamczyk, J. J., M. R. Williams, J. T. Reed, D. W. Hubbard, and D. D. Hardee. 2003. Spatial and temporal occurrence of beet armyworm (Lepidoptera: Noctuidae) moths in Mississippi. *Fla. Entomol.* 86: 229–232.
- Anderson, P., M. Sadek, M. Larsson, B. Hansson, and G. Thöming. 2013. Larval host plant experience modulates both mate finding and oviposition choice in a moth. *Anim. Behav.* 85: 1169–1175.
- Atapour, M., and S. Moharrampour. 2014. Cold hardiness process of beet armyworm larvae, *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Crop. Prot.* 3: 147–158.
- Bale, J.S., G. J., Masters, I. D., Hodkinson, C., Awmack, T. M., Bezemer, V. K., Brown, J., Butterfield, A. Buse, J. C. Coulson, and J. Farrar. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biol.* 8: 1–16.
- Bates, L., R. P. Waldren, and I. D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil.* 39: 205–207.
- Bernfeld, P. 1955. Amylase,  $\alpha$  and  $\beta$ . *Method. Enzymol.* 1: 149–154.
- Biancardi, E., L. W. Panella, and R. T. Lewellen. 2012. *Beta Maritima: The Origin of Beets*, p. 293. Springer, New York.
- Bradford, M. A. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.
- Brewer, M. J., and J. T. Trumble. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 82: 1520–1526.
- Chen, Y., J. R. Ruberson, and D. M. Olson. 2008. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. *Entomol. Exp. Appl.* 126: 244–255.
- Chi, W., T. Shi, Y. Wu, and Y. Yang. 2013. Insecticide resistance status of field strains of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. *J. Econ. Entomol.* 106: 1855–1862.
- Collins, D. P., and B. Jacobsen. 2003. Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet *Cercospora* leaf spot. *Biol. Control.* 26: 153–161.
- Coyle, D. R., K. E. Clark, K. F. Raffa, and S. N. Johnson. 2011. Prior host feeding experience influences ovipositional but not feeding preference in a polyphagous insect herbivore. *Entomol. Exp. Appl.* 138: 137–145.
- Danks, H. V. 1987. Insect dormancy: an ecological perspective. *Biological Survey of Canada Monograph Series No. 1*, Ottawa.
- Davidson, C. B., J. E. Johnson, K. W. Gottschalk, and R. L. Amateis. 2001. Prediction of stand susceptibility and gypsy moth defoliation in coastal plain mixed pine hardwoods. *Can. J. For. Res.* 31: 1914–1921.
- Dybdahl, M. F., and A. Storfer. 2003. Parasite local adaptation: red queen versus suicide king. *Trends Ecol. E.* 18: 523–530.
- Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science.* 265: 1084–1086.
- Elpidina, E. N., K. S. Vinokurov, V. A. Gromenko, Y. A. Rudenshaya, Y. E. Dunaevsky, and D. P. Zhuzhikov. 2001. Compartmentalization of proteinases and amylases in *Nauphoeta cinerea* midgut. *Arch. Insect Biochem. Physiol.* 48: 206–216.
- Farahani, S., A. A. Talebi, and Y. Fathipour. 2011. Life cycle and fecundity of *Spodoptera exigua* (Lep.: Noctuidae) on five soybean varieties. *J. Entomol. Soc. Iran.* 30: 1–12.
- Foss, L. K., and L. K. Riese. 2003. Species-specific differences in oak foliage affect preference and performance of gypsy moth caterpillar. *Entomol. Exp. Appl.* 108: 87–93.
- Golikhajeh, N., B. Naseri, and J. Razmjou. 2016. Effect of geographic population and host cultivar on demographic parameters of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). *J. Asia-Pac. Entomol.* 19: 743–751.
- Greenberg, S. M., T. W. Sappington, B. C. Legaspi, T. X. Liu, and M. Setamou. 2001. Feeding and life history of *Spodoptera exigua* (Lepidoptera: Noctuidae) on different host plants. *Ann. Entomol. Soc. Am.* 94: 566–575.
- Harrington, R., R. A. Fleming, and I. P. Woiwod. 2001. Climate change impacts on insect management and conservation in temperate regions: can they be predicted? *Agric. Forest. Entomol.* 3: 233–240.
- Irwin, J. T., and R. E. Lee. 2000. Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *J. Insect Physiol.* 46: 655–661.
- Karimi-Malati, A., Y. Fathipour, A. A. Talebi, and M. Bazoubandi. 2014. Life table parameters and survivorship of *Spodoptera exigua* (Lepidoptera: Noctuidae) at constant temperatures. *Environ. Entomol.* 43: 795–803.
- Khatab, H., and I. Khatab. 2005. Responses of Eucalypt trees to insect feeding (gall-forming psyllid). *Int. J. Agric. Biol.* 7: 979–984.
- Konarev, V. G. 1996. Problems of species and genome in plant breeding, pp. 14–24. *In* A. V. Konarev (ed.), *Molecular Biological Aspects of Applied Botany, Genetics and Plant Breeding. Theoretical Bases of Plant Breeding*. VIR, St. Petersburg, Russia.
- Lawrence, P. K., and K. R. Koundal. 2002. Plant protease inhibitors in control of phytophagous insects. *Electron. J. Biotechnol.* 5: 1–17.
- Layton, M. B. 1994. The 1993 beet armyworm outbreak in Mississippi and future management guidelines, pp. 854–856. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.
- Lee, K. P., S. T. Behmer, D. Raubenheimer, and S. J. Simpson. 2002. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). *J. Insect Physiol.* 48: 655–665.
- Li, Z. L., S. G. Wei, B. Wei, Z. Y. Liang, H. J. Yang, and S. H. Wang. 2005. Effects of five host plants on development, reproduction and activities of esterase of the beet armyworm (*Spodoptera exigua*). *Chin. Bull. Entomol.* 42: 284–289.
- Lu, Z. Q., and Y. H. Xu. 1998. The consideration of the incessant outbreak of the cotton bollworm, *Helicoverpa armigera*. *Entomol. Knowl.* 35: 132–136.
- Mehrkhou, F. 2013. Effect of soybean varieties on nutritional indices of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae). *Afr. J. Agric. Res.* 8: 1528–1533.
- Mehrkhou, F., M. Mousavi, and A. A. Talebi. 2015. Effect of different solanaceous host plants on nutritional indices of *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Crop. Prot.* 4: 329–336.
- Mello, M. O., and M. C. Silva-Filho. 2002. Plant-insect interactions: an evolutionary arms race between two distinct defense mechanisms. *Braz. J. Plant Physiol.* 14: 71–81.

- Merrill, R. M., D. Gutiérrez, O. T. Lewis, J. Gutiérrez, S. B. Díez, and R. J. Wilson. 2008. Combined effects of climate and biotic interactions on the elevational range of a phytophagous insect. *J. Anim. Ecol.* 77: 145–155.
- Meyer, G. A., and M. E. Montgomery. 2004. Relationship between leaf age and the food quality of cottonwood foliage for the gypsy moth, *Lymantria dispar*. *Oecologia* 72: 527–532.
- Mitchell, E. R. 1979. Migration by *Spodoptera exigua* and *S. frugiperda*, pp. 386–393. In R. L. Rabb, J. S. Kennedy (eds.), *Movement of Highly Mobile Insects: Concepts and Methodology in Research*. North Carolina State University, Raleigh, NC.
- Pauchet, Y., A. Muck, A. Svatos, D. G. Heckel, and S. Preiss. 2008. Mapping the larval midgut lumen proteome of *Helicoverpa armigera*, a generalist herbivorous insect. *J. Proteome. Res.* 7: 1629–1639.
- Parmesan, C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biol.* 13: 1860–1872.
- Prokopy, R. J., and W. J. Lewis. 1993. Application of learning to pest management, pp. 308–42. In D. R. Papaj and A. C. Lewis (eds.), *Insect Learning: Ecological and Evolutionary Perspectives*. Chapman & Hall, NY.
- Rauscher, M. D. 1982. Strain differentiation in *Euphydryas editha* butterflies: larval adaptation to different hosts. *Evolution*. 36: 581–590.
- SAS Institute. 1989. *SAS/STAT User's Guide*, Fourth Edition, Cary NC.; SAS Institute Inc.
- Serrano, R., and R. Gaxiola. 1994. Microbial models and salt stress tolerance in plants. *Crit. Rev. Plant. Sci.* 13: 121–138.
- Shaghfi, M., and O. Valizadegan. 2014. Study the effects of pyridalyl on larvae of *Spodoptera exigua* (Hübner) at first, second and third ages during 72 hours in laboratory conditions. *Int. J. Curr. Microbiol. Appl. Sci.* 3: 310–315.
- Shah-Smith, D. A., and R. G. Burns. 1997. Shelf-life of a biocontrol *Pseudomonas putida* applied to the sugar beet seeds using commercial coatings. *Biocontrol. Sci. Technol.* 7: 65–74.
- Singh, O. P., and S. B. B. Parihar. 1988. Effect of different hosts on the development of *Heliothis armigera* (Hübner). *Bull. Entomol.* 29: 168–172.
- Singer, M. C., and C. S. McBride. 2009. Multitrait, host-associated divergence among sets of butterfly strains: implications for reproductive isolation and ecological speciation. *Evolution*. 64: 921–933.
- Showler, A. T., and P. J. Moran. 2003. Effects of drought stressed cotton, *Gossypium hirsutum* L. on beet armyworm, *Spodoptera exigua* (Hübner) oviposition and larval feeding preferences and growth. *J. Chem. Ecol.* 29: 1997–2011.
- Sivakumar, S., M. Mohan, O. L. Franco, and B. Thayumanavan. 2006. Inhibition of insect pest  $\alpha$ -amylases by little and finger millet inhibitors. *Pest. Biochem. Physiol.* 85: 155–160.
- Smits, P. H., M. C. vanVelden, M. van de Vrie, and J. M. Valk. 1987. Feeding and dispersion of *Spodoptera exigua* larvae and its relevance for control with a nuclear polyhedrosis virus. *Entomol. Exp. Appl.* 43: 67–72.
- Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. *Seasonal Adaptations of Insects*. Oxford University Press, Oxford.
- Telang, A., V. Booton, R. F. Chapman, and D. E. Wheeler. 2001. How female caterpillars accumulate their nutrient reserves. *J. Insect Physiol.* 47: 1055–1064.
- Terra, W. R., and C. Ferreira. 1994. Insect digestive enzymes: properties, compartmentalization and function. *Comp. Biochem. Physiol.* 109: 1–62.
- Terriere, L. 1984. Induction of detoxification enzymes. *Annu. Rev. Entomol.* 29: 71–88.
- Thompson, J. N. 2005. *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago, IL.
- Thomson, L. J., S. Macfadyen, and A. A. Hoffmann. 2010. Predicting the effects of climate change on natural enemies of agricultural pests. *Biol. Control* 52: 296–306.
- Wang, M., C. C. Wu, and F. X. Zhu. 2003. Studies on insecticide susceptibility of the beet armyworm fed on different host plants. *J. Plant Protec.* 30: 193–197.
- Wang, Y., Q. N. Cai, Q. W. Zhang, and Y. Han. 2006. Effect of the secondary substances from wheat on the growth and digestive physiology of cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Eur. J. Entomol.* 103: 255–258.
- Williams, G. C. 1966. *Adaptation and natural selection*. Princeton University Press, Princeton.
- Wu, C. C., and M. Wang. 2003. Effect of different diets on the susceptibility of insecticides and the activity of detoxifying enzymes in the beet armyworm, *Spodoptera exigua*. *Chin. J. Pest Sci.* 5: 56–60 (in Chinese).
- Vazquez-Arista, M., R. H. Smith, N. A. Martinez-Gallardo, and A. Blanco-Labra. 1999. Enzymatic differences in the digestive system of the adult and larva of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *J. Stored Prod. Res.* 35: 167–174.
- Zhang, B., H. S. Helen, J. J. Wang, and H. Liu. 2011a. Performance and enzyme activity of beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) under various nutritional conditions. *Agric. Sci. China* 10: 737–746.
- Zhang, B., H. Liu, and H. S. Helen. 2011b. Effect of host plants on development, fecundity and enzyme activity of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Agric. Sci. China* 10: 1232–1240.
- Zhu-Salzman, K., J. L. Bi, and T. X. Liu. 2005. Molecular strategies of plant defense and insect counter-defense. *Insect Sci.* 12: 3–15.