OXFORD

Digestive Physiology and Nutritional Responses of *Autographa gamma* (L.) (Lepidoptera: Noctuidae) on Different Sugar Beet Cultivars

Bahram Naseri[1,2], Neshat Golikhajeh[1], and Foroogh Rahimi Namin[1]

[1]Department of Plant Protection, Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, Ardabil, Iran and
[2]Corresponding author, e-mail: Bnaseri@uma.ac.ir

Abstract

Digestive enzymatic activity and nutritional responses of *Autographa gamma* (L.) (Lepidoptera: Noctuidae), an important insect pest of sugar beet, on nine sugar beet cultivars (Peritra, Karolina, Paolita, Lenzier, Tiller, Ardabili, Persia, Rozier, and Dorothea) were studied. The highest proteolytic activity of fourth and fifth instar of *A. gamma* was in larvae fed on cultivar Persia. The highest amylolytic activity of fourth and fifth instar was observed in larvae fed on cultivars Rozier and Dorothea, respectively. The lowest proteolytic and amylolytic activities in fourth instar were observed on cultivar Tiller; whereas the lowest activities in fifth instar were detected on cultivars Karolina and Tiller, respectively. Larval weight in both larval instars (fourth and fifth) was the heaviest on cultivar Persia and the lightest on cultivar Karolina. Furthermore, weight gain of larvae was the highest on cultivar Persia and the lowest on cultivar Karolina. The results of this study suggest that cultivar Tiller was the most unsuitable host plant for feeding of *A. gamma*.

Key words: *Autographa gamma*, sugar beet, enzymatic activity, feeding response

Introduction

Sugar beet [*Beta vulgaris* (L.)] is known as one of the important industrial crops in Iran (Sadeghi et al. 2010) and many countries around the world (Shah-Smith and Burns 1997; Collins and Jacobson 2003; Biancardi et al. 2012). It has a variety of insect pests from different orders and families (Hein et al. 2009). The silver Y moth, *Autographa gamma* (L.) (Lepidoptera: Noctuidae), as a polyphagous insect pest, is known as one of the economically important pests of sugar beet in Iran and many parts of the world (Kheyri 1989; CAB 2003; Keyhanian et al. 2005). The larvae of *A. gamma* damage sugar beet plants by defoliating, and consequently reducing crop yields (Novák 1975).

In polyphagous insects, the quality of host plants can affect the larval growth, longevity, and fecundity of the adult (Bernays and Chapman 1994). Host plant resistance is often the first line of defense against herbivorous insect pests because of minimized pesticide application on this host plant, leading to reduced environmental and human health risks (Hein et al. 2009). Population dynamics of *A. gamma* can be influenced by climatic condition and host plant type (Maceljski and Balarin 1974). It is noticeable that the development of this pest on an unfavorable food source may take three times longer than favorable food sources (Honek et al. 2002).

Because of the important biochemical role of digestive enzymes such as proteases and α-amylases in insect growth, when the activity of these enzymes is inhibited, the insect nutrition is impaired (Kaur and Gupta 2015). Also, it is noticeable that these enzymes' activities are associated with the nature of food sources or chemical compounds ingested by insects (Mendiola-Olaya et al. 2000). The quantity and type of food, temperature, and midgut pH are important factors directly influencing the digestive enzymatic activity and providing the energy requirement for growth and development of insects (Sivakumar et al. 2006). For the development of new management strategies against herbivorous insects, it is necessary to study their feeding performance and function of digestive enzymes (Lawerence and Koundal 2002).

Several studies have recently been done about the effect of various host plants on digestive enzymatic activity and nutritional responses of lepidopteran larvae (Naseri et al. 2010; Hemati et al. 2012; Mehrkhou 2013; Rahimi Namin et al. 2014; Mardani-Talaee et al. 2014; Hosseininejad et al. 2015; Teimouri et al. 2015); however, no published research articles are available regarding digestive physiology and nutritional responses of *A. gamma* on either sugar beet cultivars or other host plants. Accordingly, the objective of this research was to study the effect of different sugar beet cultivars on the feeding rate, and digestive proteolytic and amylolytic activities of *A. gamma*. We expect that the results of this research will provide practical information for designing comprehensive pest management strategies against *A. gamma*.

Materials and Methods

Sugar Beet Sources

Seeds of nine sugar beet cultivars (Peritra, Karolina, Paolita, Lenzier, Tiller, Ardabili, Persia, Rozier, and Dorothea) were obtained from the Plant and Seed Modification Research Institute of Sugar Beet (Ardabil, Iran). They were grown in the research field of the University of Mohaghegh Ardabili (Ardabil, Iran) in May 2014. The selected cultivars were the most commercially cultivated sugar beets in different regions of Iran. For this study, the young leaves of sugar beet cultivars (in the eight leaf stage) were transferred to a growth chamber at $25 \pm 1\,°C$, $65 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D) h and were used for larval feeding.

Rearing of Insect

Originally, the fifth instar larvae of *A. gamma* were collected from sugar beet fields of Northern Khorasan, Iran. Nine separate stock cultures were reared, for two generations, on each sugar beet cultivar before being used in the experiments. The first and second instars were simultaneously reared until the third instar, and then they were divided into the individual plastic plates (8 cm in diameter, depth 1 cm). The insects were kept inside a growth chamber at the same conditions noted in 'Sugar Beet Sources' section.

Insect Dissection and Preparation of Sample

Larval rearing methods for this section were similar to those mentioned in the 'Rearing of insect'. Fourth and fifth instars of *A. gamma* fed on each sugar beet cultivar (five replicates per 30 larvae sampled per cultivar) were dissected under dissecting microscope in distilled ice water. The midguts were separated and homogenized with a handling homogenizer. The samples were centrifuged at 13,000 rpm for 10 min at 4 °C, and then the resulting supernatants of each tube were pooled and stored at $-20\,°C$ until use.

Protein Determination of *A. gamma* Larvae

The method of Bradford (1976) was used for determining general protein concentrations in the midgut of fourth and fifth instar, using bovine serum albumin (BSA) (Roche Co., Germany) as the standard.

Assay for Proteolytic Activity of *A. gamma*

Azocasein substrate (Sigma Chemical Co., St Louis, USA) was used for proteolytic assay (Elpidina et al. 2001) with minor modification. The reaction mixture containing 80 µl of 1.5% azocasein solution in sodium phosphate-borate buffer (0.05 M, pH 12) was mixed with 50 µl of the enzyme extract and incubated at 37 °C for 50 min. The proteolysis was terminated by the addition of 100 µl 30% trichloroacetic acid (TCA) as a stopper, and then rested at 4 °C for 30 min. Precipitations were reached by centrifugation of 13,000 rpm for 10 min at 4 °C. Then 100 µl of supernatant was mixed with 100 µl of 2 M NaOH, and the absorbance was read at 440 nm. In the blanks, TCA was added to the mixture before adding the enzyme extract. All experiments were carried out in three replicates, and each experiment was repeated at least three times.

Assay for Amylolytic Activity of *A. gamma*

The amylolytic activity assay was carried out according to the Bernfeld (1955) method, using 1% soluble starch (Sigma Chemical Co., St Louis, USA) as a substrate and dinitrosalicylic acid (DNS) (Sigma Chemical Co., St Louis, USA.) as a stopper. Twenty microliters of the enzyme extract were incubated for 30 min at 37 °C with 500 µl of succinate-glycine-2, morpholinoethan sulfonic acid buffer (0.01 M, pH 10) and 40 µl of soluble starch. The assay was terminated by the addition of 100 µl DNS and keeping in boiling water for 10 min. The absorbance was read at 540 nm after cooling on ice. All experiments were performed in three replicates, and each experiment was repeated at least three times.

Protein and Starch Determination of Sugar Beet Cultivars

Protein content in the leaves of each sugar beet cultivar was quantified using BSA as a standard according to Bradford (1976). Two hundred milligrams of the leaves of each tested cultivar were homogenized in 10 ml of distilled water. Then, 100 µl of the homogenate was mixed with 3 ml of Bradford reagent (Sigma Chemical Co., St Louis, USA). The samples were incubated in darkness at 37 °C and absorbance was read at 595 nm.

Starch content in the leaves of sugar beet cultivars was determined by the method of Bernfeld (1955) using starch as the standard. Two hundred milligrams of the leaves of each sugar beet cultivar were homogenized in 35 ml of distilled water and heated to boiling point. Then 100 ml of each sample was mixed with 2.5 ml of iodine reagent, including 0.02% I_2 (Maarssen Co., Holland) and 0.2% KI (Merck Co., Germany), and the absorbance was read at 580 nm.

Nutritional Responses of *A. gamma*

A gravimetric method described by Waldbauer (1968) was used to determine the nutritional responses (larval weight, food consumed, feces produced and weight gain) of the fourth and fifth instar of *A. gamma* on nine sugar beet cultivars (30 replicates for each cultivar). Nutritional responses were calculated on the dry weight basis. The leaves of each sugar beet cultivar were weighed, and then transferred into the individual plastic plates (8 cm in diameter, depth 1 cm) for larval feeding. The initial weight of the newly ecdysed fourth and fifth instar was recorded and then they were reared on the weighed leaves of each cultivar for 24 h. The remnant leaves, after 24 h, were weighed and replaced with fresh leaves. Weight of food ingested is subtracted by the weight of remnant leaf from the whole weight of leaf supplied to the larvae. The produced feces were collected and weighed at the end of each experiment. To achieve the percentage of dry weights of the larvae (fourth and fifth instar), leaves and produced feces, 20 extra specimens for each were weighed, dried in an oven (at 60 °C for 48 h), and then re-weighed.

Analysis of Data

The data were analyzed separately for each experiment with one-way ANOVA followed by comparison of the means with LSD test at $\alpha = 0.05$ using statistical software Minitab 16.0 (www.minitab.com). Before analysis, all data were examined for normality using Kolmogorov-Smirnov test, which were normally distributed.

Results

Assay for Proteolytic Activity of *A. gamma*

The highest proteolytic activity of fourth instar ($F = 9.88$; $df = 8, 18$; $P < 0.01$) was recorded on cultivar Persia; whereas the lowest activity was observed on cultivar Tiller. Also, the highest proteolytic activity of fifth instar ($F = 14.38$; $df = 8, 18$; $P < 0.01$) was on cultivar Persia, and the lowest activity was on cultivar Karolina (Fig. 1).

Assay for Amylolytic Activity of *A. gamma*

The fourth instar ($F = 19.09$; $df = 8, 18$; $P < 0.01$) fed on cultivars Rozier and Persia showed the highest amylolytic activity; whereas
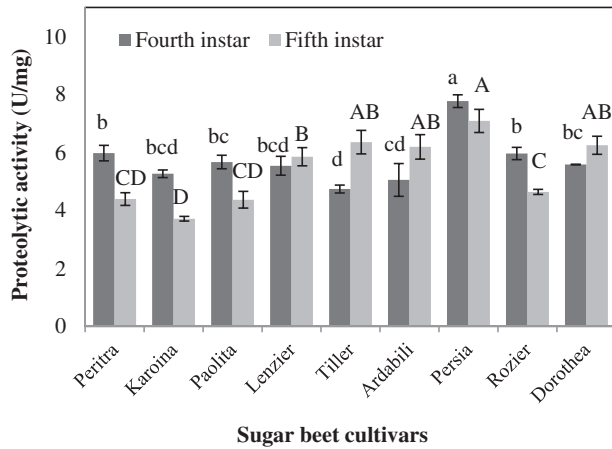
Fig. 1. Mean (± SE) general proteolytic activity (U/mg) of midgut extracts from fourth and fifth instar *A. gamma* fed on different sugar beet cultivars. The means followed by different upper case letters (fifth instar) or by different lower case letters (fourth instar) are significantly different from the other means in the same instar (*P* < 0.01, LSD).
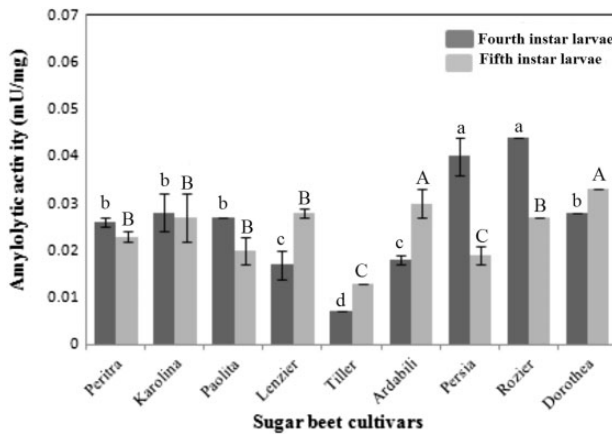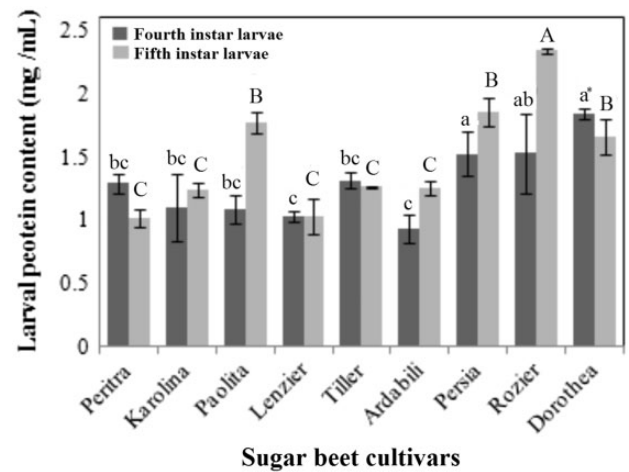

Fig. 3. Mean (± SE) protein content of midgut extracts from fourth and fifth instar *A. gamma* fed on different sugar beet cultivars. The means followed by different upper case letters (fifth instar) or by different lower case letters (fourth instar) are significantly different from the other means in the same instar (*P* < 0.01 and *P* < 0.05*, LSD).

Fig. 2. Mean (± SE) amylolytic activity (mU/mg) of midgut extracts from fourth and fifth instar *A. gamma* fed on different sugar beet cultivars. The means followed by different upper case letters (fifth instar) or by different lower case letters (fourth instar) are significantly different from the other means in the same instar (*P* < 0.01, LSD).
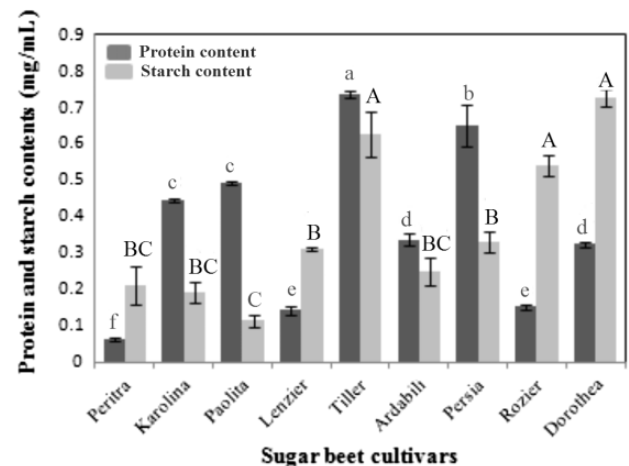

Fig. 4. Mean (± SE) protein and starch contents in the leaves of different sugar beet cultivars. The means followed by different upper case letters (starch content) or by different lower case letters (protein content) are significantly different (*P* < 0.01, LSD).

the lowest activity was in larvae fed on cultivar Tiller. The amylolytic activity of fifth instar (*F* = 5.51; df = 8, 18; *P* < 0.01) was the highest on cultivars Dorothea and Ardabili, and the lowest on cultivars Persia and Tiller (Fig. 2).

Protein Determination of *A. gamma* Larvae
The fourth instar (*F* = 3.25; df = 8, 18; *P* < 0.05) fed on cultivars Dorothea and Persia showed the highest protein content, while the lowest content was on cultivars Ardabili and Lenzier. The protein content of fifth instar (*F* = 26.14; df = 8, 18; *P* < 0.01) was the highest on cultivar Rozier and the lowest on cultivars Peritra, Lenzier, Ardabili, Tiller, and Karolina (Fig. 3).

Protein and Starch Determination of Sugar Beet Cultivars
Our data indicated significant differences in protein (*F* = 116; df = 8, 18; *P* < 0.01) and starch (*F* = 12.10; df = 8, 18; *P* < 0.01) contents in the leaves of different sugar beet cultivars (Fig. 4). The highest

and lowest protein contents were recorded for cultivars Tiller and Peritra, respectively. The content of starch was the highest in cultivars Dorothea, Tiller and Rozier, and the lowest in cultivar Paolita.

Nutritional Responses of *A. gamma*
The highest larval weight of fourth instar (*F* = 110.97, df = 8, 194, *P* < 0.01) was detected when larvae were fed on cultivar Lenzier, while the lowest value was on cultivar Ardabili. Moreover, the highest and lowest values of food consumption (*F* = 8.58, df = 8, 211, *P* < 0.01) were on cultivars Ardabili and Peritra, respectively. The results showed that the larvae fed on cultivar Persia produced the highest feces (*F* = 24.45, df = 8, 202, *P* < 0.01) compared with the other cultivars. In addition, the highest weight gain of larvae (*F* = 84.55, df = 8, 209, *P* < 0.01) was recorded on cultivar Ardabili and the lowest on cultivar Paolita (Fig. 5).
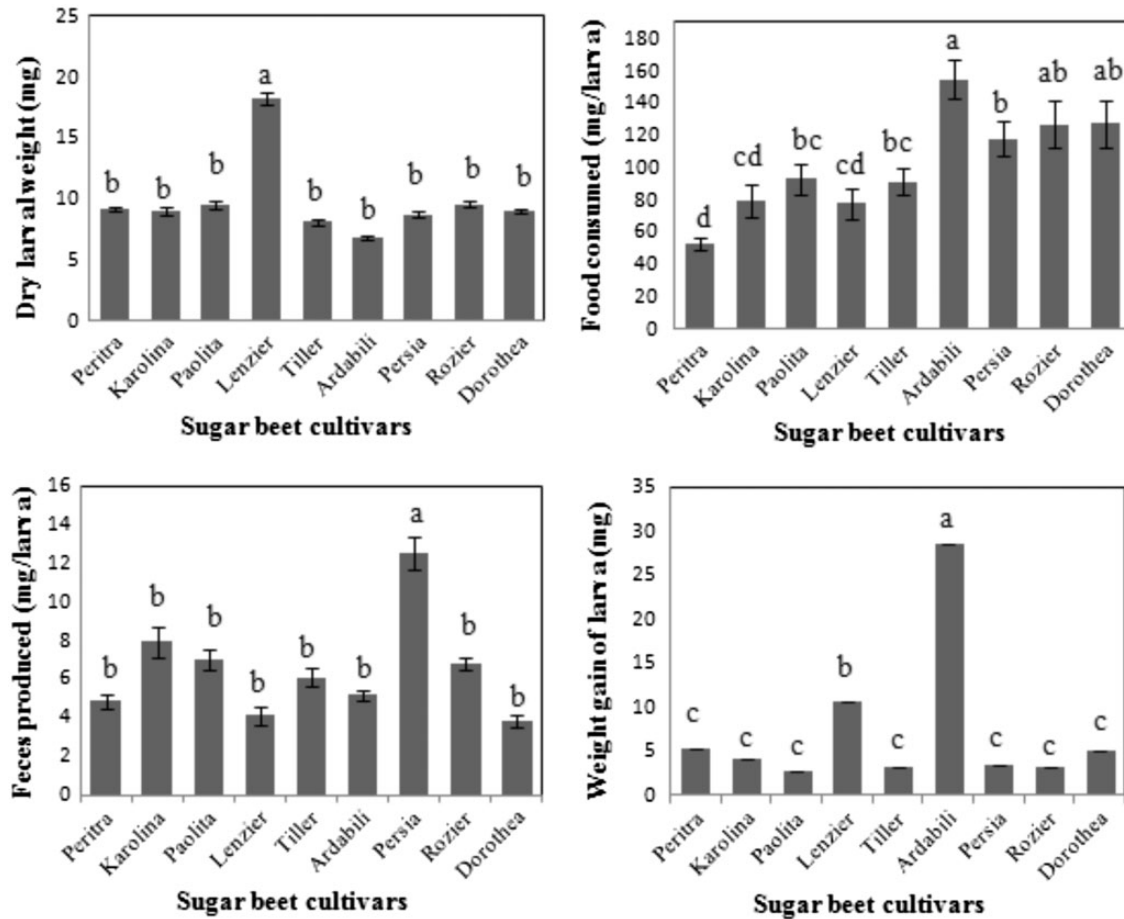
Fig. 5. Mean (± SE) dry larval weight, food consumed, feces produced, and larval weight gain of fourth instar *A. gamma* fed on different sugar beet cultivars. The means followed by different letters are significantly different ($P < 0.01$, LSD).

The highest larval weight of fifth instar ($F = 497.29$, df $= 8, 201$, $P < 0.01$) was on cultivar Persia, while the lowest value was on cultivar Karolina. The highest and lowest food consumption ($F = 20.72$, df $= 8, 217$, $P < 0.01$) was on cultivars Rozier and Ardabili, respectively. Also, feces produced ($F = 16.53$, df $= 8, 207$, $P < 0.01$) was the highest on Peritra and the lowest on cultivar Ardabili. In addition, the highest larval weight gain of *A. gamma* ($F = 57.80$, df $= 8, 199$, $P < 0.01$) was detected on cultivar Persia, while the lowest value was on cultivar Karolina (Fig. 6).

The highest and lowest larval weight of both (fourth and fifth) instar ($F = 484.47$, df $= 8, 203$, $P < 0.01$) were detected on cultivars Persia and Karolina, respectively. The fourth and fifth instar reared on cultivar Rozier consumed more food than those reared on the other cultivars ($F = 13.37$, df $= 8, 217$, $P < 0.01$). The highest amount of feces was produced ($F = 16.62$, df $= 8, 204$, $P < 0.01$) by larvae fed on cultivar Peritra. The weight gain of larvae ($F = 49.23$, df $= 8, 197$, $P < 0.01$) was the highest when they were reared on cultivar Persia, and the lowest on cultivar Karolina (Fig. 7).

Discussion

Host plant is an important factor in regulating insect populations (Umbanhowar and Hastings, 2002). For identification of crop resistant to herbivorous insects, one way is to study the biological and

physiological attributes of insect pests reared on various host crops (Sarfraz et al. 2006). In addition, the study of host plant resistance is an important tool for identifying the antidigestive compounds in the plant that can be useful in integrated pest management (Lewis et al. 1997).

Compatible to other works (Hemati et al. 2012; Naseri et al. 2014; Rahimi Namin et al. 2014), the findings of this research showed that the digestive physiology of *A. gamma* larvae was significantly affected by different tested sugar beet cultivars. The highest proteolytic activity of fourth and fifth instar was on cultivar Persia (Fig. 1), which could be due to higher food consumption by the larvae or the presence of some plant proteinase inhibitors (PIs). It was reported that the insects can quickly modify their midgut enzyme compositions by up-and down-regulations of protease synthesized in response to PIs (Jongsma and Bolter 1997; Harsulkar et al. 1999). As the presence of PIs in host plant can inhibit gut proteases of insects and may prevent larval growth (Hilder 1987; Johnston et al. 1993), the lowest proteolytic activity of *A. gamma* larvae on cultivars Tiller (in fourth instar) and Karolina (in fifth instar) (Fig. 1) may be due to the presence of secondary metabolites or PIs in these cultivars.

The activity of digestive amylases is dependent on the type of food diet (Shekari et al. 2008). According to the results of this study (Fig. 2), *A. gamma* larvae fed on cultivar Rozier (in fourth instar) and Dorothea (in fifth instar) showed the highest amylolytic activity.
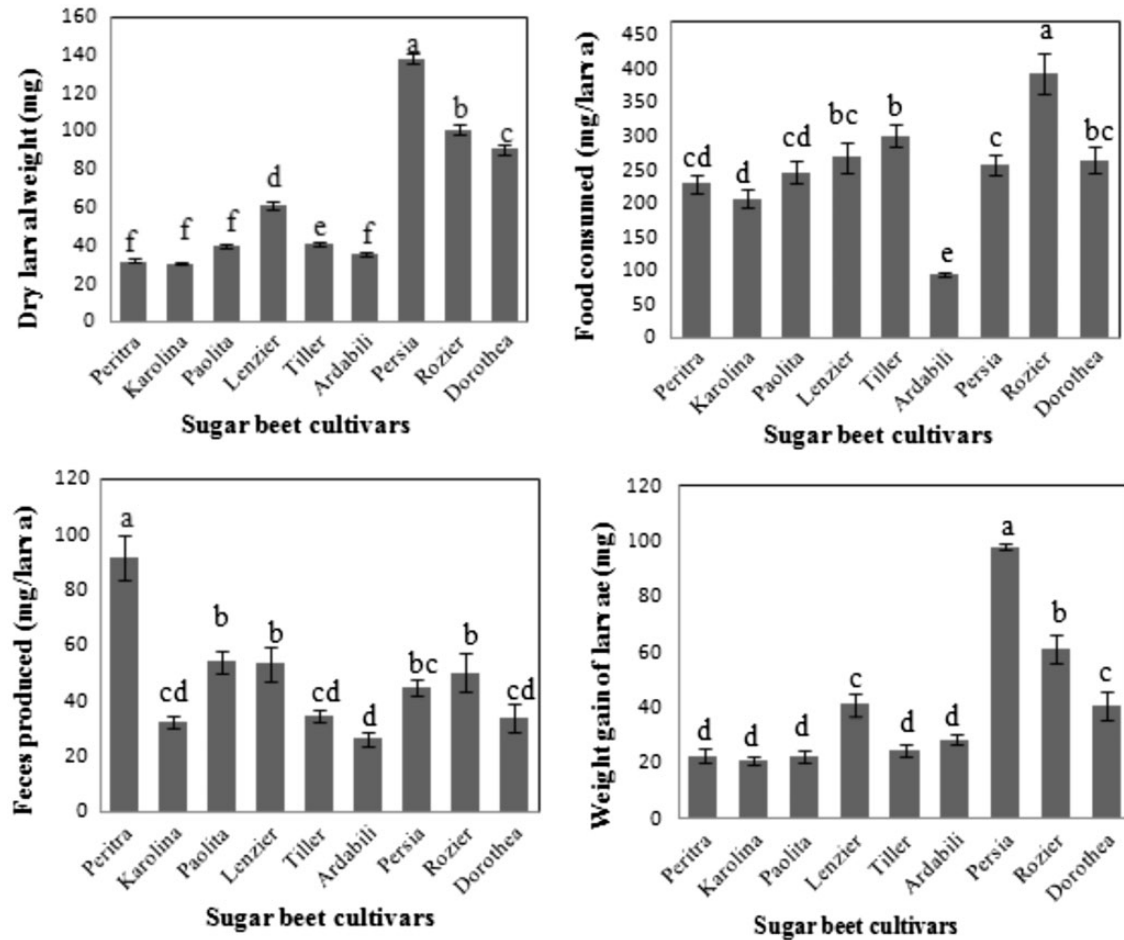
Fig. 6. Mean ($\pm$ SE) dry larval weight, food consumed, feces produced, and larval weight gain of fifth instar *A. gamma* fed on different sugar beet cultivars. The means followed by different letters are significantly different ($P < 0.01$, LSD).

It is generally accepted that food contents have important role in regulating the level of digestive amylases of insects (Sarate et al. 2012). Because variations in the starch content in sugar beet cultivars can lead to differences in the amylolytic activity of *A. gamma*, thus, the highest amylolytic activity in larvae fed on cultivars Rozier and Dorothea is attributed to the highest starch content of these cultivars.

In lepidopteran insects, the proportion of protein and carbohydrate in the diets can influence the larval performance and digestive enzymatic activity (Sarate et al. 2012; Rahimi Namin et al. 2014). In our study, fourth instar of *A. gamma* fed on cultivar Tiller showed the lowest protease activity. Moreover, although cultivar Tiller had the highest protein and starch contents, the lowest amylolytic activity of fourth and fifth instar was observed on this cultivar. Such reduced enzymatic activity in *A. gamma* larvae fed on Tiller (with a high protein and starch content) might be due to the presence of some secondary biochemicals in this cultivar, which should be confirmed by additional tests. Although the quantity of protein and starch in a diet can affect the optimal development of herbivores, the nutritive quality of dietary macronutrients, especially proteins, also has a significant effect on plant-insect interactions (Felton 1996; Chen 2007; Kotkar et al. 2009). Therefore, it is possible that cultivar Tiller was nutritionally unbalanced host that negatively influenced digestive enzyme activity of *A. gamma*. According to data achieved from midgut protease and amylase activities, and the

protein and starch contents of the nine tested sugar beet cultivars, it seems that lepidopteran insects are able to evaluate the macromolecule content in the food and adjust the synthesized digestive enzymes (Kotkar et al. 2009).

Body weight is one of the main biological indices of insect population dynamics (Liu et al. 2004). The highest larval weight and weight gain of larvae in fifth and both larval instars were on cultivar Persia. As the proteolytic and amylolytic activities of larvae and protein concentration in larval midgut were the highest on cultivar Persia, it could be suggested that this cultivar is a suitable host plant for feeding and increasing body weight of larvae. To confirm this result, our personal observations regarding biological attributes of *A. gamma* showed that the highest pupal weight and lowest larval mortality was detected when *A. gamma* was reared on cultivar Persia. The results of this study showed that the larval weight and larval weight gain of both instars were relatively low on cultivar Tiller. Moreover, lower pupal weight and higher larval mortality of *A. gamma* on cultivar Tiller (data not shown) indicate that this cultivar is unsuitable host plant for feeding of *A. gamma*.

The highest food consumed in both larval instar of *A. gamma* was on cultivar Rozier, whereas cultivar Rozier had relatively lower content of protein. It is possible that lower dietary protein in this cultivar led to increased food consumption by *A. gamma* larvae. In phytophagous insects, the nutritional value of a diet can influence the larval growth, survival, and development and fecundity of the
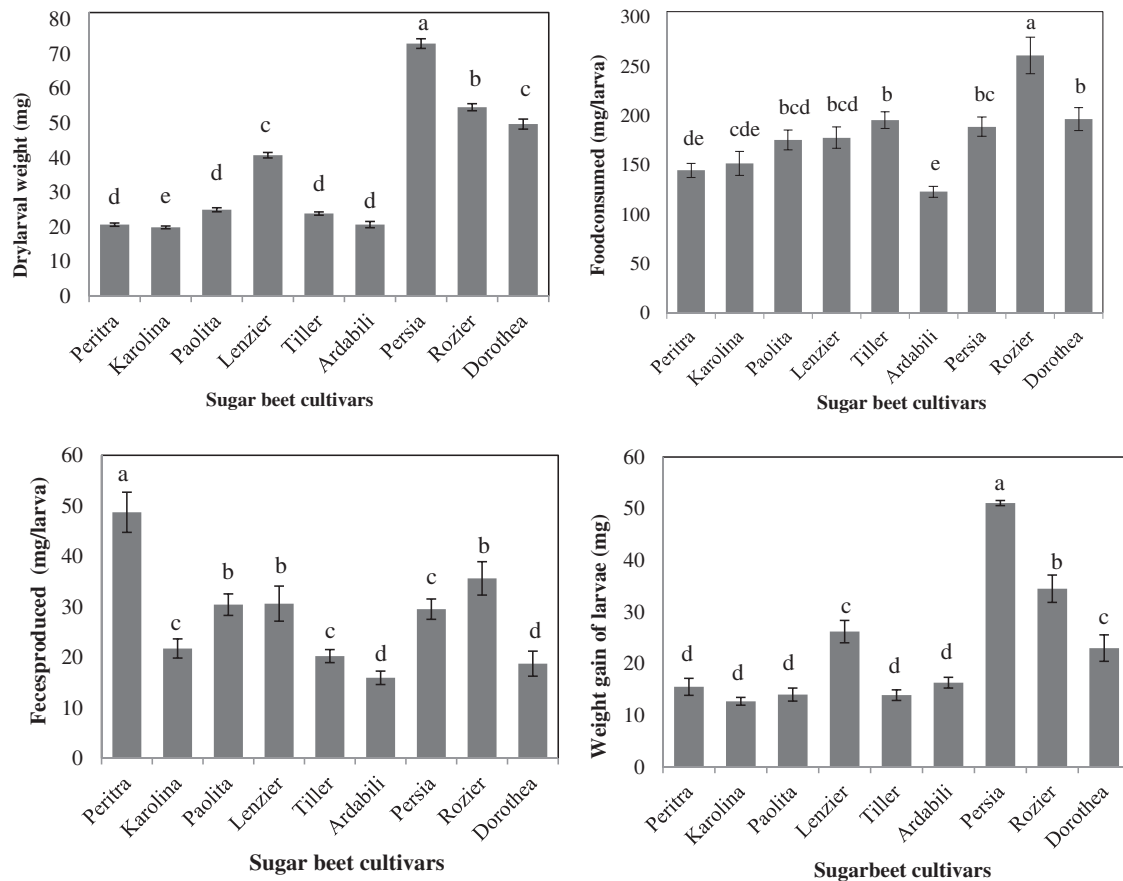
Fig. 7. Mean (± SE) dry larval weight, food consumed, feces produced, and larval weight gain of both (fourth and fifth) instar *A. gamma* fed on different sugar beet cultivars. The means followed by different letters are significantly different (*P* < 0.01, LSD).

adult (Bernays and Chapman 1994). Some factors such as the amount and nature of food consumed could affect the availability of nutrients and the efficacy of converting food to body mass (Barton Browne and Raubenheimer 2003).

As seen in Figures 5 and 6, nutritional responses of fourth- and fifth-instar larvae were considerably different with each other. For example, fourth instar larvae of *A. gamma* consumed more food when they were reared on cultivar Ardabili; whereas fifth instar larvae consumed less food when they were fed on this cultivar. The dietary requirements of phytophagous insects change during development, especially in penultimate and ultimate instars, and such changes are commonly reflected in changes of food consumption (Barton Browne and Raubenheimer 2003). In a lepidopteran larva, the nutritional requirements over different developmental periods are positively correlated with the mass of the insect (Phillipson 1981). Moreover, differences in digestive ability of lepidopteran larvae can affect nutritional performances of penultimate and ultimate instars (Patankar et al. 2001, Lazarevic et al. 2004). For example, although older larvae may digest their food less completely than younger ones, their digestive enzymatic activities are more fully developed for growth and development (Dhillon and Sharma 2004). In addition, differences in physiological changes during penultimate and ultimate instars are probably another reason for the differences in nutritional responses of these two larval instars on various sugar beet cultivars.

According to the outcomes of our observations, among sugar beet cultivars tested, lower efficiency of conversion of digested food for both larval instars (fourth and fifth instar), lower pupal weight,

and higher larval mortality were on cultivar Tiller. Moreover, studying life history of *A. gamma* on different sugar beet cultivars, Golikhajeh et al. (2016) reported that the longest larval development was on cultivar Tiller. In the current study, the lowest digestive enzymatic activity of the larvae were on this cultivar as well, indicating that the larvae fed on cultivar Tiller had lower abilities to convert the digested food into their body biomass.

Conclusions

According to generated data in this study, *A. gamma* food source was associated with digestive enzyme activity. By combining the results from a study on the life cycle of *A. gamma* (Golikhajeh et al. 2016) on different sugar beet cultivars and the results of the current research, we conclude that cultivar Persia is a suitable (susceptible) and cultivar Tiller an unsuitable (resistant) host plant for *A. gamma*. In the future studies, the characterization and extraction of secondary metabolites of resistant sugar beet cultivars will largely assist in designing of practical strategies in integrated pest management programs.

Acknowledgments

References Cited

Barton Browne, L. B., and D. Raubenheimer 2003. Ontogenetic changes in the rate of ingestion and estimates of food consumption in fourth and fifth instar *Helicoverpa armigera* caterpillars. J. Insect Physiol. 49: 63–71.

Bernays, E. A., and R. F. Chapman 1994. Host-plant Selection by Phytophagous Insects. Chapman & Hall, New York, NY.

Bernfeld, P. 1955. Amylases, *a* and *b*. Methods Enzymol. 1: 149–54.

Biancardi, E., L. W. Panella, and R. T. Lewellen 2012. Beta Maritima: the Origin of Beets. 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: 248–54.

CAB. 2003. Crop protection compendium: global module. Common wealth Agricultural Bureau International, Wallingford, UK.

Chen, H., E. Gonzales-Vigil, C. G. Wilkerson, and G. A. Howe 2007. Stability of plant proteins in the gut of insect herbivores. Plant Physiol. 143: 1954–67.

Collins, D. P., and B. Jacobsen 2003. Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet Cercospora leaf spot. Biol. Control. 26: 153–61.

Dhillon, M. K., and P. D. Sharma 2004. Studies on biology and behavior of *Earias vittella* (Lepidoptera: Noctuidae) for mechanisms of resistance in different cotton genotypes. Crop Protect. 23: 235–41.

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: 206–16.

Felton, G. W. 1996. Nutritive quality of plant protein: sources of variation and insect herbivore responses.Arch. Insect Biochem. Physiol. 32: 107–30.

Golikhajeh, N., B. Naseri, and F.R. Namin. 2016. Demographic parameters of *Autographa gamma* (Noctuidae) affected by sugar beet cultivars. J. Lepid. Soc. (in press).

Harsulkar, A. M., A. P. Giri, A. G. Patankar, V. S. Gupta, M. N. Sainani, P. K. Ranjekar, and V. V. Deshpande. 1999. Successive use of non-host plant proteinase inhibitors required for effective inhibition of gut proteinases and larval growth of *Helicoverpa armigera*. J Plant Physiol. 121: 497–506.

Hein, G. L., M. A. Boetel, and L. D. Godfrey. 2009. Part IV. Major insect and arthropod pests. *In* R. M. Harveson, L. E. Hanson, G. L. Hein (eds.), Compendium of Beet Diseases and Pests, 95–117. APS Press, St. Paul, USA.

Hemati, S. A., B. Naseri, G. Nouri Ganbalani, H. Rafiee Dastjerdi, and A. Golizadeh. 2012. Digestive proteolytic and amylolytic activities and feeding responses of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on different host plants. J. Econ. Entomol. 105: 1439–46.

Hilder, V. A., A. M. R. Gatehouse, S. E. Sheerman, R. F. Barker, and D. Boulter. 1987. A novel mechanism of insect resistance engineered into tobacco. Nature 330: 160–5.

Honek, A., V. Jarosic, Z. Martinkova, and I. Novak. 2002. Food induced variation of thermal constants of development and growth of *Autographa gamma* (Lepidoptera: Noctuidae) larvae. Eur. J. Entomol. 99: 241–52.

Hosseininejad, A. S., B. Naseri, and J. Razmjou. 2015. Comparative feeding performance and digestive physiology of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae-fed 11 corn hybrids. J. Insect Sci. 15: 1–6.

Johnston, K.K.A., J. A. Gatehouse, and J. H. Anstee. 1993. Effects of soybean trypsin inhibitors on the growth and development of larval *Helicoverpa armigera*. J. Insect Physiol. 39: 657–64.

Jongsma, M. A., and C. J. Bolter. 1997. The adaptation of insects to plant protease inhibitors. J. Insect Physiol. 43: 885–95.

Kaur, R., and A. K. Gupta. 2015. Insect amylase-plant amylase inhibitor interaction is key to success of transgenics against insectherbivory. Biochem. Anal. Biochem. 4: 201.

Keyhanian, A. A., M. Taghi Zadeh, M. V. Taghaddosi, and Y.K. Zadeh. 2005. A faunistic study on insect pests and its natural enemies in canola fields at different regions of Iran. Pajouhesh Sazandegi 68: 2–8.

Kheyri, M. 1989. An inventory of pests attacking sugar beet in Iran. Entomol. Phytopathol. Appl. 56: 1–2.

Kotkar, H. M., P. J. Sarate, V. A. Tamhane, V. S. Gupta, and A. P. Giri. 2009. Responses of midgut amylases of *Helicoverpa armigera* to feeding on various host plants. J. Insect Physiol. 55: 663–70.

Lawerence, P. K., and K. R. Koundal. 2002. Plant proteinase inhibitors in control of phytophagous insects. Electr. J. Biotechnol. 5: 93–109.

Lazarevic, J., V. Peric-Mataruga, M. Vlahovic, and M. Mrdakovic. 2004. Effects of rearing density on larval growth and activity of digestive enzymes in *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Folia Biologica. 52: 1–2.

Lewis, W. J., J. C. van Lenteren, S. C. Phatak, and J. H. I. I. I. Tumlinson. 1997. A total system approach to sustainable pest management. Proc. Natl. Acad Sci. USA. 94: 12243–8.

Liu, Z., D. Li, P. Y. Gong, and K. J. Wu. 2004. Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), on different host plants. Environ. Entomol. 33: 1570–6.

Maceljski, M., and I. Balarin 1974. Factors influencing the population density of the looper *Autographa gamma* L. in Yugoslavia. Acta Entomol. Jugoslavica 10: 63–76.

Mardani-Talaee, M., V. Rahimi, and A. Zibaee. 2014. Effects of host plants on digestive enzymatic activities and some components involved in intermediary metabolism of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae). J. Entomol. Acarol. Res. 46: 96–101.

Mehrkhou, F. 2013. Effect of soybean varieties on nutritional indices of beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae). Afr. J. Agric. Res. 8: 1528–33.

Mendiola-Olaya, E., A. Valencia-Jimenez, S. Valdes-Rodriguez, J. Delano-Frier, and A. Blanco-Labra. 2000. Digestive amylase from the larger grain borer, *Prostephanus truncatus* Horn. Comp. Biochem. Physiol. B. 126: 425–33.

Naseri, B., Y. Fathipour, S. Moharramipour, and V. Hosseininaveh. 2010. Nutritional indices of the cotton bollworm, *Helicoverpa armigera*, on 13 soybean varieties. J. Insect Sci. 10: 151 available online: insectscience.org/ 10.151.

Naseri, B., D. Kouhi, J. Razmjou, and A. Golizadeh. 2014. Digestive enzymatic activity and nutritional responses of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae fed various tomato cultivars. J. Econ. Entomol. 107: 1655–61.

Novák, I. 1975. Critical number of *Autographa gamma* L. caterpillars (Lep., Noctuidae) on sugar-beet. Sbornik UVTI-Ochrana Rostlin. 11: 295–9.

Patankar, A. G., A. P. Giri, A. M. Harsulkar, M. N. Sainani, V. V. Deshpande, P. K. Ranjekar, and V. S. Gupta. 2001. Complexity in specificities and expression of *Helicoverpa armigera* gut proteinases explains polyphagous nature of the insect pest. Insect Biochem. Mol. Biol. 31: 453–64.

Phillipson, J. 1981. Bioenergetic options and phylogeny, pp. 20–45. *In* C. R. Townsend, and P. Calow (eds.), Physiological Ecology: An Evolutionary Approach to Resource Use. Blackwell Scientific, Oxford.

Rahimi Namin, F., B. Naseri, and J. Razmjou. 2014. Nutritional performance and activity of some digestive enzymes of the cotton bollworm, *Helicoverpa armigera*: response to bean cultivars. J. Insect Sci. 14: 93.

Sadeghi, H., S., Eshraghi, and L., Behne 2010. A contribution to the fauna of weevils (Col.: Curculionidae) associated with sugar beet fields in North–east of Iran. Munis Entomol. Zool. 5: 753–7.

Sarate, P. J., V. A. Tamhane, H. M. Kotkar, N. Ratnakaran, N. Susan, V. S. Gupta, and A. P. Giri. 2012. Developmental and digestive flexibilities in the midgut of a polyphagous pest, the cotton bollworm, *Helicoverpa armigera*. J. Insect Sci. 12: 42 available online: insectscience.org/12.42.

Sarfraz, M., L. M. Dosdall, and B. A. Keddie. 2006. Diamondback moth-host plant interactions: implications for pest management. Crop Protect. 25: 625–36.

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.

Shekari, A., J. J. Sendi, K. Etebari, A. Zibaee, and A. Shadparvar. 2008. Effect of *Artemisia annua* L. (Astracea) on nutritional and enzyme activities of elm

leaf beetle *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomelidae). Pest. Biochem. Physiol. 91: 66–74.

Sivakumar, S., M. Mohan, O. L. Franco, and B. Thayumanavan. 2006. Inhibition of insect pest α-amylases by little and finger millet inhibitors. Pest. Biochem. Physiol. 85: 155–60.

Teimouri, N., J. J. Sendi, A. Zibaee, and R. Khosravi. 2015. Feeding indices and enzymatic activities of carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: pyrallidae) on two commercial pistachio cultivars and an artificial diet. J. Saudi Soc. Agric. Sci. 14: 76–82.

UmbanhowarJ., and A. Hastings. 2002. The impact of resource limitation and the phenology of parasitoid attack on the duration of insect herbivore outbreaks. J. Theor. Popul. Biol. 62: 259–69.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229–88.