Journal of the Saudi Society of Agricultural Sciences (2015) **14**, 76–82

King Saud University

Journal of the Saudi Society of Agricultural Sciences

www.ksu.edu.sa
www.sciencedirect.com

FULL LENGTH ARTICLE

Feeding indices and enzymatic activities of carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: pyrallidae) on two commercial pistachio cultivars and an artificial diet

Naeimeh Teimouri, Jalal Jalali Sendi *, Arash Zibaee, Roya Khosravi

University of Guilan, Faculty of Agriculture, Department of Plant Protection, 41635-1314 Rasht, Iran

Received 22 March 2013; accepted 23 August 2013
Available online 31 August 2013

KEYWORDS

Ectomyelois ceratoniae;
Pistachio;
Feeding indices;
Lipase;
Amylase;
Protease

Abstract Feeding indices and enzymatic activities of *Ectomyelois ceratoniae* (Zeller) were studied in a growth chamber under controlled conditions (29 ± 2 °C, relative humidity of 70 ± 5% and a photoperiod of 16:8 (L:D) hours) on two commercial Pistachio cultivars (Akbari and Kalequchi) and an artificial diet. Feeding indices of *E. ceratoniae* larvae differed significantly on three hosts ($P < 0.05$). The relative consumption rate was calculated to be 5.36 ± 0.009, 11.10 ± 1.49 and 10.631 ± 0.599 (mg/mg/day) on artificial diet, Akbari and Kalequchi cultivars, respectively. Carob moth larvae reared on Akbari cultivar showed the highest efficiency of conversion of digested food (ECD) (5.64 ± 0.43). The highest amount of efficiency of conversion of ingested food (ECI) was obtained on artificial diet but approximate digestibility (AD) was the lowest on this diet. The highest enzymatic activities of alpha-amylase, general proteases and lipase were observed in the midgut of larvae reared on artificial diet. Total protein and lipid value were highest in larvae that were reared on artificial diet.

1. Introduction

Wild pistachio (*Pistacia vera* L.) belongs to the sumac (Anacardiaceae) plants. The genus *Pistacia* has 11 species, all of which secrete turpentine oil. Various pests attack Pistachio out of which carob moth is considered as the most serious one. Halperin (1986) and Rice (1978)reported damage of/caused by *Ectomyelois ceratoniae* (Zell) and *Apomyelois transitella* Wal. on Pistachio.

The carob moth *E. ceratoniae* (Zeller), also known as date moth, is an important pest attacking fruit trees and nut crops throughout the world. It is also a major field pest of pomegranate, *Punica granatum* L., date, *Phoenix dactylifera* L. and almond, *Prunus dulcis* (Mill.) (Norouzi et al., 2008). Adult carob moths begin to emerge early in May in Iran and preferably attack pomegranate. Apparently, pomegranate

* Corresponding author. Tel.: +98 66 908 17.
E-mail address: jjalali@guilan.ac.ir (J.J. Sendi).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

1658-077X © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.jssas.2013.08.003

fruits provide suitable conditions for oviposition of these moths. After completing some generations on pomegranate, they attack Pistachio (Mehrnejad, 1992). This insect is able to continue its damage during storage. Its maximum activity is found to be during September and November in Rafsanjan, a main region of Pistachio cultivation in Iran. Adult moths cannot attack un-cracked hull nuts, however the larvae can penetrate into un-cracked shell from stem end. It appears that the carob moth spends several generations on alternate hosts, mainly pomegranate before attacking pistachio nuts (Mehrnejad, 1992).

Metabolic efficiency of insect feeding on plant varieties (Waldbauer, 1968), and the effect of plant on insect metabolism and interactions between insects and their food sources are shown by using feeding indices (Bhat and Bhattacharya, 1987). For example in *Spodoptera frugiperda* Smith (Lep.: Noctuidae) feeding indices were calculated on 9 bermuda grass types and based on these results, resistance and susceptible varieties were distinguished (Jamjanyn and Quisenberry, 1988). Feeding indices demonstrate the digestion efficiency or utilization of diet or diet ingredients and in fact illustrate the conversion of food to the biomass of insects. These indices can provide valuable information about the positive or negative impact of ingredients or total food (Cohen, 2005). The general feeding indices used are: approximate digestibility (AD), Efficiency of conversion of ingested food (ECI), Efficiency of digested food (ECD) and relative Consumption rate (RCR) (Waldbauer, 1968). One of the easiest methods of control of carob moth is the use of resistant varieties (Shakeri, 2004). Feeding indices may be used as the methods for establishing resistant varieties.

Growth of insect is influenced by biotic and abiotic factors such as temperature, humidity, food quality and quantity (Jansen and Groot, 2004). These factors will also affect insect physiological processes. So the activity of digestive enzymes depends on the nature of food and chemicals ingested (Mendiola-Olaya et al., 2000; Silva et al., 2009). Digestive enzymes are commonly found in the salivary secretions and various regions of the digestive tract of insects. Digestive enzymes play a major role in the body of insects by converting complex food materials into smaller molecules necessary to provide energy and metabolites (Wigglesworth, 1984).The major digestive enzymes in the midgut of insects consist of amylases, lipases and proteases that are similar in their hydrolytic nature. α-Amylases are the hydrolytic enzymes that catalyze the hydrolysis of α-D-(1,4)-glucan linkages in glycogen and other related carbohydrates (Franco et al., 2000). Lipases catalyze the hydrolysis of fatty acid ester bonds, and are widely distributed among animals, plants and microorganisms (Grillo et al., 2007). Peptidases act on peptide bonds and include endopeptidases and exopeptidases (Terra and Ferreira, 2005).

Because of the economic importance of *E. ceratoniae* as a pest species, there has been a considerable amount of research on various aspects of its developmental biology. In the present study, we have investigated some basic measures of fitness on field hosts and artificial diet. For a better control and improved management strategy, a better understanding of its digestive physiology is helpful, which hopefully will lead to new strategies for management of this important pest.

2. Materials and methods

2.1. Insect rearing

Approximately 1000 infested pomegranate fruits by carob moth larvae were originally collected from pomegranate orchards of agricultural research center of Yazd city, Yazd province (Center of Iran) and were transported to the laboratory. Then the larvae of carob moth were separated from infested fruits. They were reared in transparent plastic jars ($30 \times 20 \times 13$ cm) on Akbari and Kalequchi cultivars and artificial diet (containing wheat flour 72 g, honey 12, glycerin 10 g, yeast 1 g, distilled water 5 ml) in controlled condition (29 ± 2 °C, 70 ± 5% RH, and 16:8 L:D photoperiod). The emerged adults from infested fruits were transferred into transparent jars (18×7 cm) and were provided with cotton wool soaked in 10% honey for feeding. The carob moths were reared on each diet in the laboratory for three generations before the experiments.

2.2. Feeding efficiency

Newly ecdysed fifth instar larvae were collected from the stock culture and transferred into plastic jars $30 \times 20 \times 13$ cm with a hole covered by a fine mesh net for ventilation, and containing artificial diet or Akbari or Kalequchi cultivars. Experiments were carried out for 3 days. The experiments were conducted with eight replicates for each diet. A gravimetric technique was used to determine weight gain, food consumption, and the amount of feces produced. The newly ecdysed fifth instar larvae were starved 4 h prior to the start of experiments to exude gut contents. Nutritional indices were measured on the dry weight basis. After measuring the weight of the fifth instar larvae, they were introduced to each host diet, and the weights of the larvae were recorded before and after feeding until they stopped feeding. Efficiency indices were calculated as described by Huang and Ho (1998):

Approximate digestibility (AD) = 100 (E – F)/F, Efficiency of conversion of ingested food (ECI) = 100 P/E, Efficiency of digested food (ECD) = 100 P/(E – F), Relative Consumption rate (RCR) = E/TA, where: A = dry weight of larvae in the start of experiment, E = dry weight of consumed food, F = dry weight of produced feces, P = dry weight of the biomass of larvae, T = duration of the experiment (3 days).

2.3. Preparation for enzymatic activities

The fifth instar larvae (2 days old) were dissected under a stereomicroscope in an ice-cold saline buffer (0.15 M NaCl). Their midguts were removed from the insect body, rinsed in ice cold distilled water. Then placed in a pre-cooled homogenizer with a known volume of distilled water and ground before centrifugation. The homogenates were then centrifuged at 13,000 rpm for 20 min at 4 °C. The resulting supernatants were transferred to new micro tubes and frozen at −20 °C until used.

2.4. Assay for α-amylase activity

α-Amylase activity was assayed according to Bernfeld (1955), by dinitrosalicylic acid (DNS) as the reagent and 1% soluble