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Bioecology of *Nephopterygia austeritella* (Lep.: Pyralidae), a potential biological control agent of *Prosopis farcta* (Fabaceae) in central Iran

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Summary *Prosopis farcta* (Fabaceae) is a native and common perennial weed plant in Iran. In search of environmental-friendly control methods against *P. farcta,* we discovered the seed feeder moth *Nephopterygia austeritella* (Lepidoptera; Pyralidae) in central Iran and studied its bioecology for the first time from 2008 through 2009. Infestation pattern, larval feeding behaviour, developmental period, seasonal occurrence and the adverse impact of the moth on the reproductive organs of *P. farcta* were investigated. Diagnostic morphological characters of the fifth larval instar of *N. austeritella* are provided. Two gregarious ectoparasitoids were reared and identified as *Apanteles subcamilla* and *Phanerotoma leucobasis* (Hymenoptera: Braconidae). Mortality rates of the larvae were 3.03 and 13.44% in 2008 and 2009, respectively. Larvae destroyed 29.6-38.4% of the pods of their host plants. The potential of *N. austeritella* as an efficient biological control agent in IPM programs against *P. farcta* is discussed.

Additional keywords: mesquite, impact, parasitoid, pest, seed, weed.

Introduction

Syrian mesquite, *Prosopis farcta* (Banks & Solander) (Fabaceae), is a perennial, thorny, xerophilous and salt-tolerant shrub which is widely spread from India to Algeria between latitudes *ca* 10° (in Yemen) and 50° in Kazakhstan (Bazzaz, 1973; Bisby *et al.,* 2011). *Prosopis farcta* is an economically multifaceted plant. It has been regarded as a useful plant for fixation of nitrogen and the production of nutrient-rich pods and foliage, especially in saline and arid environments and serves as a source of fodder in many countries (Said *et al.*, 2002; Dogan *et al.,* 2004; Omidi *et al.*, 2012). However, special biological attributes of *P. farcta* have increased the competitive and prevalent properties of this weed in orchards (e.g. olive and temperate-zone fruits) and fields (e.g. sesame and vegetables) (Johnson, 1983; Pasiecznik *et al.,* 2004; Sertkaya *et al.,* 2005; Qasem, 2007) such as its deep rhizobia-symbiont root system (Bazzaz, 1973; Canadell *et al.,* 1996; Atomov and Aktoklu, 2007; Fterich *et al.,* 2011) that propagates through both long-lived seeds and rhizome buds (Qasem, 2007). These features allow *P. farcta* to produce enormous and durable dense stands and quickly become a dominant weed in agroecosystems. *Prosopis farcta* is also a host of witches' broom disease which is the most destructive disease of alfalfa in Iran (Esmailzadeh-Hosseini *et al.,* 2011).

In order to control *P. farcta* and other species of the same genus in agricultural ecosystems, efforts were made to preserve the beneficial attributes of these plants while limitting their dispersal and competition with agricultural crops. To date, soil solarisation, mechanical methods and chemical control (Qasem, 2007) have failed to effectively control *P. farcta*. By contrast, biological agents can be used against *P. farcta* in an integrated management program (Johnson, 1983; Mc Kay and Gandolfo, 2007; Qasem,

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2007). Among the insects associated with *P. farcta*, the seed beetle *Caryedon angeri* Semenov (Coleoptera: Chrysomelidae) is reported as the most harmful agent attacking the reproductive organs of *P. farcta* in the Middle East (Johnson, 1983; Sertkaya *et al.*, 2005). *Caryedon angeri* usually infests *Acacia* spp. and *P. farcta*, but there are concerns about its tendency to attack non-target species, such as groundnut (*Arachis hypogaea* L. (Fabaceae) (Bagheri-Zenous, 1992; Anton and Delobel, 2004). It is argued that *C. angeri* is not a good candidate for the biological control of the weed (Sertkaya *et al.,* 2005). Parasitoid braconid wasps attacking lepidopteran species of the families Lycaenidae, Geometridae and Gelechiidae that feed on *P. farcta* were recorded by Halperin (1986).

Recent observations on *P. farcta* shrubs in southern Iran revealed that the pyralid moth *Nephopterygia austeritella* Amsel (Lepidoptera: Pyralidae, Phycitinae) can feed on the pods of *P. farcta* (Alipanah *et al.,* 2012). *Nephopterygia austeritella* has been recorded from Sudan (Amsel, 1965) to the Canary Islands (Spain), Egypt (Asselbergs, 2009) and Iran (Alipanah *et al.,* 2012). This work was intended to study the bioecology of *N. austeritella* under natural conditions in central Iran and its negative impact on the reproductive organs of *P. farcta*.

Materials and methods

Infestation by the herbivore

The infestation of *P. farcta* by *N. austeritella* was studied in an abandoned orchard of approximately 10 hectares in Yazd County (31°89´ N, 54°36´ E, 1230 m a.s.l.), Yazd province, Iran. A map showing the study site is illustrated in Figure 1. Sampling was performed according to the *P. farcta* phenology, from May (early spring coinciding with leaf formation) to November (late autumn during leaf fall) at 10-15 day intervals, during 2008 and 2009. A random sample of 100 pods was made from branches of *P. farcta*. The sampled pods were then transferred to the laboratory where they were dissected with a sharp knife. The number of infested pods, larvae and externally parasitized larvae (as in Figure 2B-F) were recorded. The phenology of the host plant was recorded in each sampling date.

Infestation pattern

Infestation pattern of pods of *P. farcta* by *N. austeritella* was examined in a sample taken on 27 June 2009 from Yazd County area coinciding with the late emergence of adults (Figure 3). Fifty stems of *P. farcta* (one stem in each bush) were randomly selected and all pods (114), the number of infested pods and larva(e) within each pod (Figure 2B-C) were recorded. Infestation pattern of pods was calculated based on single or multiple larvae in each pod.

Description of larval instars

Larval instars were documented by determining the distance between the external extreme of the ocelli as the breadth of the head capsule of the larvae (Freitas, 1993). The body length of the larvae was measured from the anterior edge of the anteclypeus to the posterior edge of the anal plate. These data were used for determining the larval instars of *N. austeritella* using Dyar's rule. All measurements were made using a calibrated ocular micrometer of an Olympus stereomicroscope on 5-10 larvae collected from the study area of Yazd County in each sampling date. The larvae are described here for the first time. The fifth larval instar was described and illustrated in detail to distinguish it from other species of the family Pyralidae. Their mouthparts were dissected following the methods of Godfrey (1972), and the setal nomenclature follows that of Hasenfuss and Kristensen (2003).

Impact of *N. austeritella* on pods of *P. farcta*

Natural impact of *N. austeritella* on *P. farcta* pods was evaluated in March 2008 and 2009 (late winter) coinciding with the end of annual growing period of the plant at three areas in central Iran: Abarkouh coun-

Figure 1. Map of sampling areas of *Nephopterygia austeritella* in central Iran.

ty, Yazd province (31°12´ N, 53°28´ E, 1510 m a.s.l.), Darab county, Fars province (28°47´ N, 54°33´ E, 1100 m a.s.l.) and Yazd county, Yazd province, Iran (Figure 1). For this purpose, several bushes of *P. farcta* were randomly selected and all pods of a single branch were selected to provide a sample of 100 pods. There were six replicates (totally 600 pods) in each area. The pods were then dissected in the laboratory and the rate of damaged pods was calculated.

Natural enemies of *N. austeritella*

We inspected the infested pods of *P. farcta* for larval parasitoids of *N. austeritella* in the study area of Yazd County. Anaesthetized or with observable parasitoid larvae were transferred to the laboratory and kept in ventilated plastic rearing boxes. Adult parasitoids were collected and identified by the third author (Tobias *et al.,* 1986; Van Achterberg, 1990).

Results

General biology

The first adults of *N. austeritella* emerged in late May (Figures 2A and 3) and their appearance lasted until mid-June. This period was synchronized with the first flowering period of *P. farcta*, which began from early May, in the studied areas, when the formation of green fruits occurs. The females laid their eggs singly on the surface of the young green pods of the host plant (Figure 2B).

The first larval instar ate the egg chorion immediately after hatching and it then

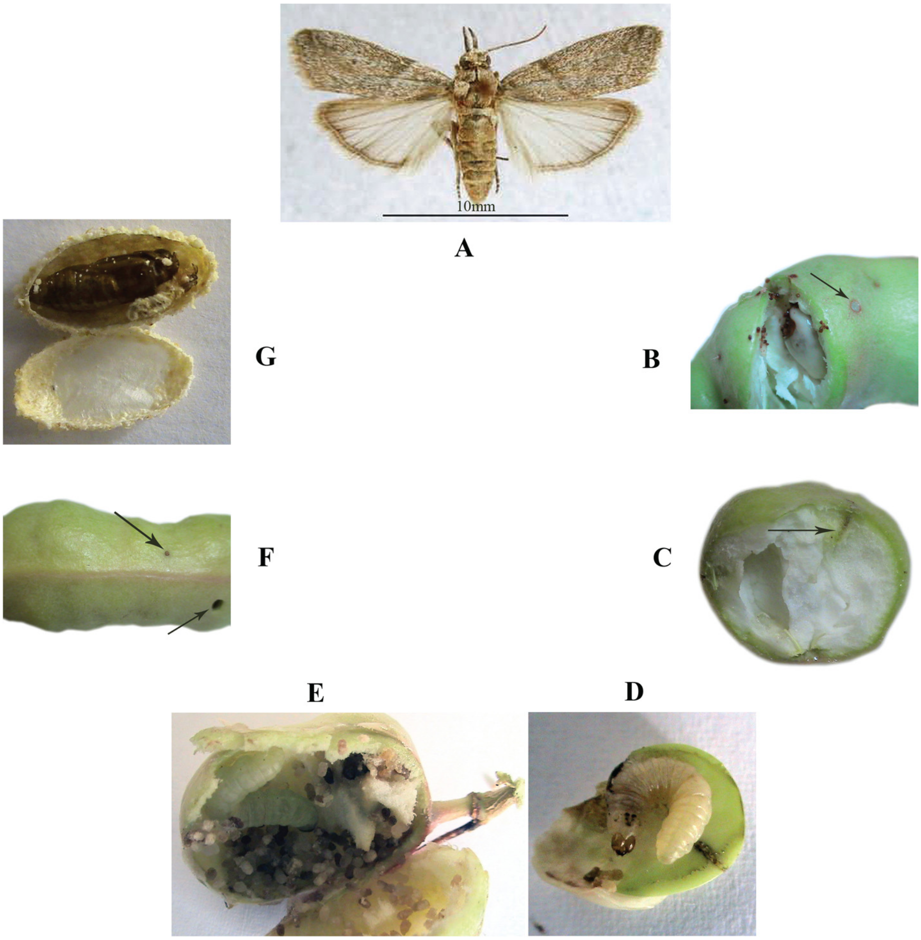

Figure 2. Life cycle of *Nephopterygia austeritella*. The adult female (A) lays its eggs on the surface of a green pod (B), the first instar larva moves to the seed in pod (C), the second instar larva feeds within the seed (D), the larva in the third and following instars completely destroys the mesocarp and seeds of the ripening pod (E), the larva makes a hole to exit from the destroyed pod (the above and below arrow indicates the position of egg and the exiting hole on the pod, respectively) (F), The last instar larva pupates within an oval silken cocoon (G).

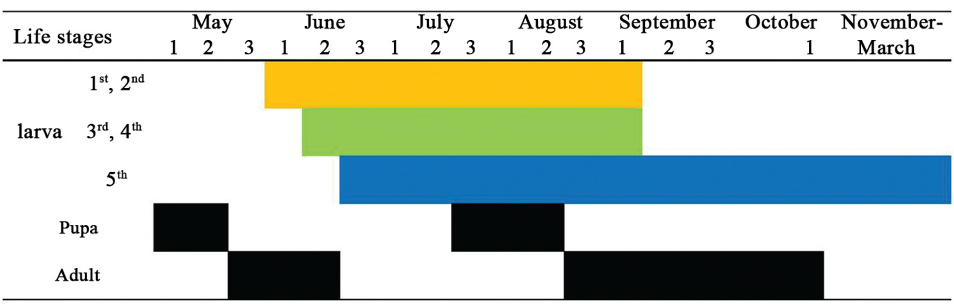

Figure 3. Phenology of *Nephopterygia austeritella* on the Syrian mesquite, *Prosopis farcta*, in central Iran.

penetrated into the pod just beneath the egg and entered the seed (Figure 2C) by chewing a tunnel through the cotyledon. The first and second instars fed on the seed (Figure 2D-E) and destroyed it completely. These two instars were observed from early June to early September (Figure 3). Third larval instar appeared in mid-June, when they left the remains of the seed to feed on the other seeds including mesocarp (internal tissues of the pod), leaving the outer shell intact (Figure 2E). To complete the larval stage, the larva had to exit the pod by making a hole (Figure 2F). After entering another pod, the larva sealed the entrance with silk fibres. Each pod usually contains one larva, although up to two larvae were rarely seen in the same pod. Movement of the larvae from one pod to another was facilitated by silk fibres. Pupation began from late June, when the last larval instar abandoned the damaged pod and descended on the surface of the soil (Figure 3). In the rearing boxes, the last larval instar spun silken cocoons 10-13 mm in length (Figure 2G), at different heights of the boxes. Adult moths emerged (at 30 ± 2° C, 20 ± 5% R. H.) about two weeks later. Emergence period was long and lasted from mid-August to mid-September in 2008 and from mid-August to early October in 2009 (Figure 3). This was the second emergence period, which indicates the second generation of *N. austeritella*.

Our sampling in late autumn and winter showed that no larvae existed in the pods of *P. farcta* at that time. The pods had very hard outer shells and could be hardly broken by larvae. Therefore, it may be that *N. austeritella* overwinters as a full-grown fifth larval instar within a cocoon outside the pod of *P. farcta*.

Infestation pattern

Counting the number of larvae (first or second instars) within a sample taken on 27 June 2009 showed that 65.91, 20.45, 11.36 and 2.27% of pods had one to four larvae, respectively.

Description of the immature stages of *N. austeritella*

Based on the measurement of the head capsule, the insect has five larval instars (Table 1) as follows:

First larval instar

Pale yellow to creamy white, with light brownish head and prothoracic plate light orange posteriorly.

Second and third instars

Nearly in the same colour as the first one.

Fourth larval instar

Head, thoracic plate, body, thoracic legs, prolegs and anal plate of the same colour and pattern as in the fifth larval instar.

Fifth larval instar in detail

Colour: Head light brownish, with pale mottled pattern, a very short coronal suture and an ellipse of six ocelli. Ocellar area dark brown, forming dark lateral patch encompassing ocelli 1 to 5; lower part of gena close to antennal region with a dark brown patch; anteclypeal region, frontal and adfrontal sclerites pale brown; labrum dark brown, notch edged with dark brown to black; mandible light brown and edged with brown distally (Figure 4E); spinneret and labial palpi light brown; antennal segments cream (Figure 4F); body creamy yellow, integument gran-

Table 1. Measurement of head capsule width and body length of larvae of *Nephopterygia austeritella* in each instar.

Larval instar	Nr of examined larvae	Head capsule width (mm± SD)	Maximum body length (mm)
First	5	0.18 ± 0.02	1.30
Second	5	0.71 ± 0.02	7.04
Third	5	1.06 ± 0.06	10.63
Fourth	6	1.19 ± 0.01	12.75
Fifth	10	1.62 ± 0.09	14.88

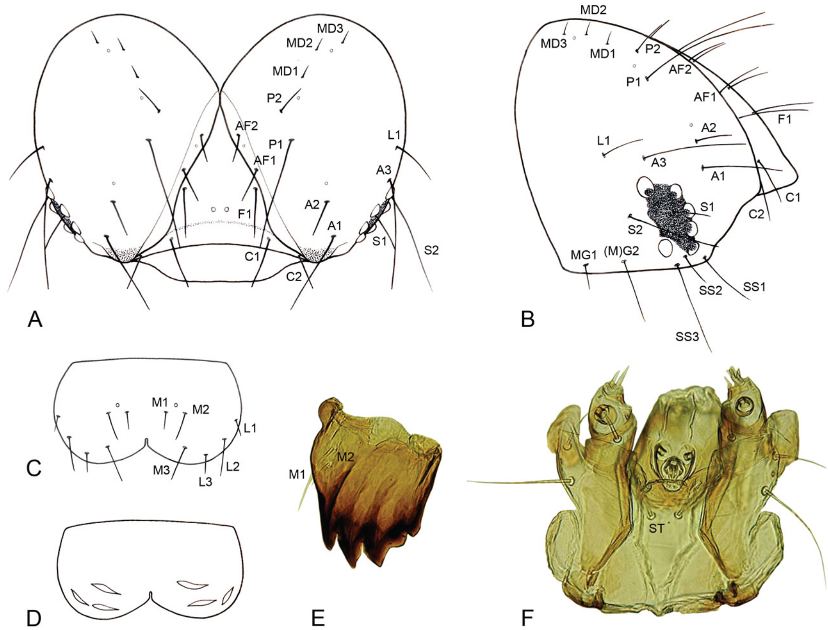

Figure 4. Head structure of the fifth larval instar of *Nephopterygia austeritella*. A) frontal view, B) lateral view, C) labrum, D) epipharynx, E) right mandible, F) maxillo-labium. Sensillium designations: A, anterior; AF, adfrontal; C, clypeal; F, frontal; L, lateral; M (in C and E designation for labral and mandibular setae); MD, microdorsal; MG, microgenal; P, parietal; S, stemmatal (= ocular, O); SS, substemmal (= subocular, SO); ST, stipular (in maxillo-labium).

ulose under low magnification; prothoracic plate very slightly darker than the ground colour, with a postero-median notch and a pattern of pale brown markings; thoracic legs yellowish cream with brownish claw (Figure 5B); anal plate creamy yellow; crochets brown; peritremes of spiracles dark.

Chaetotaxy: Head (Figure 4A–F): Frontal sclerite extended to almost four-fifths of head length, the latter slightly more than its breadth at base; adfrontals slightly tapered medially; ocellus 5 slightly extended out of the circumscribed ocellar semicircle; seta P1 almost 4 x as long as seta P2; distance between setae AF1 and AF2 nearly equal to distance between setae P1 and P2; the length of seta A2 slightly less than the length of seta A3; seta A3 almost 4 x as long as seta A1; seta S2 more than 3 x as long as seta S1.

Mouthparts: Labrum deeply notched medially (Figure 4C, D); mandible with three distinct dents along the cutting margin and two small blunt dents at the base (Figure 4E).

Thorax: (Figure 5A, C): Prothoracic plate and pre-spiracular plate separate; prothoracic plate with mottled pattern; setae XD1, XD2 and SD1 nearly equidistant from one another; seta SD1 1.2 x as long as seta XD1 and 3 x that of seta XD2; seta D2 4–5 x as long as seta D1. Seta L1 almost 3.5–4.0 x as long as seta L2; seta SV1 nearly 5 x as long as seta SV2; spiracle oval, slightly longer than the length of A1 spiracle; mesothorax and metathorax (Figure 5A): seta D2 nearly 3.5 x as long as seta D1; seta SD1 almost 5 x as long as seta SD2; setae L1, L2 and L3 equal in length and each on a separate pinaculum.

Abdomen (Figure 5A–F): Anal plate almond-shaped and more convex posteriorly; seta SD1 slightly longer than seta D2, more than 1.5 x as long as seta D1, and 3 or more times as long as seta D3. Ventral prolegs on A3 to

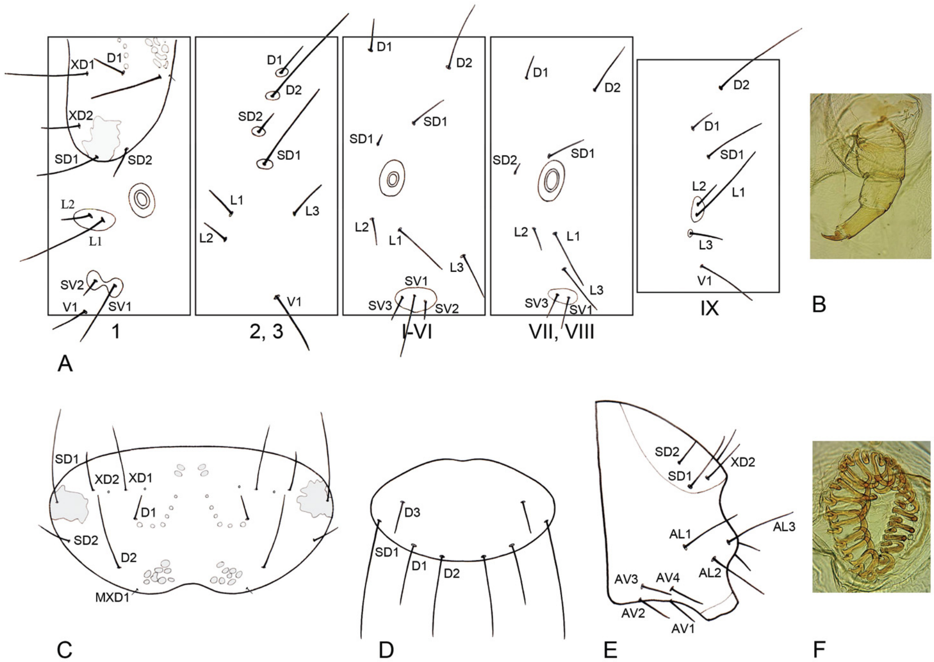

Figure 5. Diagrammatic segmental maps of the setae and sclerotizations of thorax and abdomen in the fifth larval instar of *Nephopterygia austeritella*. A) thoracic (1–3) and abdominal segments (I–IX), B) thoracic leg, C) prothoracic plate, D) anal plate, E) last (X) abdominal segment, F) crochet. Sensillium designations: A, prefix for some anal segments; dorsal, D; lateral, L; supernumary icrodorsal, MXD; subdorsal, SD; subventral, SV; ventral, V; supernumary dorsal, XD.

A6 and A10, crochets uniserial, biordinal, arranged in a complete circle (Figure 5F). In segments A1 to A8: Setae D1 and D2 nearly equidistant; seta D2 more than 2 x as long as seta D1; seta SD2 very short compared to SD1; L group trisetose (each in a unique pinaculum). SV group in A1 to A6 trisetose, while in A7 and A8 bisetose (all originate from a common pinaculum). Segment A9: seta D1 dorsal to seta D2; L group trisetose, L1 and L2 very close to each other on a common pinaculum, and L3 on a separate pinaculum; L2 more than 3.5 x as long as L1; seta L3 nearly equal in length to seta L1.

Pupa

Maximum length 6.8 mm (n=8); brown, integument almost smooth.

Impact of *N. austeritella* on *P. farcta*

Nephopterygia austeritella larvae consumed 29.6-38.4% of *P. farcta* pods in the studied areas during 2008-2009 (Table 2). All the seeds along the mesocarp of the pods were completely destroyed (Figure 2E).

Natural enemies of *N. austeritella*

From a total of 218 larvae of *N. austeritella* which were collected and reared over two years, 5 parasitized and 14 dead larvae

Table 2. Impact of *Nephoterygia austeritella* on pods of the Syrian mesquite, *P. farcta,* in central Iran.

Locality	Year	Damaged pods (%)*
Yazd	2008	32.3
	2009	36.3
Abarkouh	2008	29.8
	2009	38.4
Darab	2008	29.6
	2009	35.3

* The total number of sampled pods in all cases was 600.

Table 3. Natural enemies of *Nephopterygia austeritella* larvae in Yazd, Iran.

Year	Nr of collected larvae	Nr of parasitized larvae	Nr of dead larvae	Mortality (%)	Natural enemy
2008	99	3	------	3.03	*Apanteles subcamilla* *Phanerotoma leucobasis*
2009	119	2	------	1.68	*Phanerotoma leucobasis*
		------	13	10.92	unknown
		------	1	0.84	spider
Total	218	5	14	8.71	

were found (Table 3). The highest mortality (10.92%) of the larvae was recorded in 2009 due to an unknown factor (internal parasitoid or entomopathogen). Three and two larvae were found to be parasitized in 2008 and 2009, respectively. Two braconid parasitoids, *Apanteles subcamilla* Tobias and *Phanerotoma leucobasis* Kriechbaumer were found to parasitize the larvae of *N. austeritella* (Table 3). An unidentified spider was observed guarding its egg mass near the anaesthetized larva of *N. austeritella*.

Both parasitoid wasps were gregarious larval ectoparasitoids of *N. austeritella*. Each host larva was attacked by at most six parasitoid larvae in the pods of *P. farcta*. The last larval instar of the parasitoids underwent its pupal stage inside a white cocoon near its host body and the adult wasp emerged in September. These species were responsible for about 1.68-3.03% of mortality of *N. austeritella* larvae (Table 3).

Discussion

The present study has revealed for the first time some basic bioecological aspects of *N. austeritella* in its native habitat as a fruit feeding agent of *P. farcta*. This information will be valuable for identifying biological control agents from the genus *Prosopis* (Johnson, 1983; Mc Kay and Gandolfo, 2007). The life cycle of *N. austeritella* synchronized well with the period of fruit formation of *P. farcta*. The larvae consumed all the seeds and mesocarp of ripening pods of *P. farcta* (Figure 2E). Larval feeding resulted in a destruction of 29.6-38.4% of the pods of the plant leaving no viable seeds. Considering that each *P. farcta* pod consists of 1-9 seeds, *N. austeritella* has a larger impact on decreasing the long-lived seed bank of the plant in nature compared with the bruchid beetle, *Caryedon angeri* which consumes a fraction of the seeds of *P. farcta* (less than 50%) within a pod (Johnson, 1983; Sertkaya *et al.,* 2005).

Nephopterygia austeritella was the only pyralid moth consuming the ripening pods of *P. farcta*. Pyralids are ecologically important herbivores attacking noxious weeds (McFadyen, 1998; Blossy, 2007; Roe *et al.,* 2015). Seven species of the family Pyralidae have been universally reported targeting reproductive organs of *P. alba*, *P. glandulosa*, *P. juliflora* and *P. velutina* (Beccaloni *et al.,* 2003), six of which occur only in the New World.

As host specificity is one of the most important advantages of a biological control agent (Sheppard *et al.,* 2005; Bourchier *et al.,* 2006; Blossy, 2007), *N. austeritella* which has been yet only reported on *P. farcta*, can be considered as a promising candidate for biological control of the species of the genus *Prosopis*. Its closely related genus *Nephopterix* has a restricted host range to the species of *Euphorbia* (Cristofaro *et al.,* 1998). In North America, larvae of *Nephopterix divisella* Duponchel complete their life cycle on seven species, all in the genus *Euphorbia*. Cristofaro *et al.* (1998) considered *N. divisella* as a natural agent against two *Euphorbia* species namely, *E. milii* Desmoulins and *E. trigona* Haworth. Five species of the genus *Prosopis* are recorded from Iran, where

P. farcta, *P. koelziana* Burkart and *P. cineraria* (Linnaeus) are native and the remaining two species *P. glandulosa* Torrey and *P. juliflora* (Swartz) are introduced (Mozaffarian, 2006; Zare *et al.,* 2011). *Prosopis juliflora* is a common weed in the south of Iran (Nadjafi-Tireh-Shabankareh and Jalili, 2009). Future studies will reveal the host range and specificity of *N. austeritella* in Iran.

The Braconid parasitoids, *A. subcamilla* (Microgaterinae) and *Ph. leucobasis* (Cheloninae), attack larvae of lepidopteran species (Tobias *et al.,* 1986). *Apanteles subcamilla* has been only reported from Azerbaijan without any host record but *Ph. leucobasis* is known from Egypt, Ethiopia, Kenya, Madagascar, Nigeria, S.W. Africa, Saudi Arabia, Socotra island (Yemen), Somalia, Tanzania, Togo and Iran (Ameri *et al.,* 2012; Gadallah and Ghahari, 2013) with a wide range of hosts in the families Cosmopterygidae, Pyralidae and Gelechidae (Van Achterberg, 1990; Sobhani *et al.,* 2012; Yu *et al.,* 2012). Based on the literature, associations of these parasitoid species with *N. austeritella* and *P. farcta* are new.

Egg and pupal parasitoids of *N. austeritella* were not detected in the study, although they may be attacked by hymenopterous parasitoids (Triplehorns and Johnson, 2005). Thus, an intensive or longer period of field surveying is required to improve our knowledge of natural enemies of *N. austeritella* (Blossy, 2007). Parasitoids and other factors were responsible for the mortality of *N. austeritella* individuals during 2008 and 2009 (Table 3). Mortality factors in biological control agents of weeds have been regarded as a threat (Zalucki and Van Klinken, 2006; Zachariades *et al.,* 2011). The rate of larval parasitism of *Caryedon angeri*, a bruchid seed-beetle of *P. farcta*, by *Rhaconotus major* (Hym.: Braconidae) in Turkey varied from 62.3-100% (Sertkaya *et al.,* 2005). Parasitism of larvae and pupae of *Melipotis indomita* Walker (Lep.: Noctuidae), a biological control agent of *P. glandulosa*, was considered as an important mortality factor affecting *M. indomita* population (Cuda *et al.*, 1990). The low rate of larval parasitism of *N. austeritella* may be due to the fact that moth larvae are concealed in the pods of *P. farcta* and remain less vulnerable to the attack by parasitoids (Hill and Hulley, 1995; Van Klinken and Burwell, 2005).

N. austeritella could be used as a potential biological control agent in an integrated pest management program against *P. farcta*. Further studies would be necessary to evaluate different aspects of the biology, demography, behavior, host specificity of *N. austeritella* and its possible impact on native plants and other species of the genus *Prosopis* in Iran and neighboring countries (McFadyen, 1998; Bourchier *et al.,* 2006; Blossy, 2007).

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Βιο-οικολογία του *Nephopterygia austeritella* (Lep.: Pyralidae), ενός εν δυνάμει παράγοντα βιολογικής αντιμετώπισης του ζιζανίου *Prosopis farcta* (Fabaceae) στο κεντρικό Ιράν

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Περίληψη Το *Prosopis farcta* (Fabaceae) είναι ιθαγενές και κοινό πολυετές ζιζάνιο στο Ιράν. Σε αναζήτηση φιλικών προς το περιβάλλον μεθόδων αντιμετώπισης του *P. farcta*, εντοπίστηκε στο κεντρικό Ιράν το νυκτόβιο λεπιδόπτερο *Nephopterygia austeritella* (Lepidoptera: Pyralidae), το οποίο τρέφεται με τους σπόρους του ζιζανίου, και μελετήθηκε η βιο-οικολογία του για πρώτη φορά από το 2008 έως το 2009. Επίσης μελετήθηκαν η χωρική εξάπλωση-κατανομή της προσβολής, η διατροφική συμπεριφορά των προνυμφών, η περίοδος ανάπτυξης, η εποχιακή εμφάνιση και οι αρνητικές επιδράσεις του εντόμου στα αναπαραγωγικά όργανα του ζιζανίου. Παρουσιάζονται οι διαγνωστικοί μορφολογικοί χαρακτήρες της πέμπτης ηλικίας προνυμφών του εντόμου. Προσδιορίζονται δύο αγελαία εκτοπαρασιτοειδή του εντόμου, ως *Apanteles subcamilla* και *Phanerotoma leucobasis* (Hymenoptera: Braconidae). Τα ποσοστά θνησιμότητας των προνυμφών ήταν 3,03 και 13,44% το 2008 και το 2009, αντίστοιχα. Οι προνύμφες κατέστρεψαν το 29,6 έως 38,4% των λοβών των φυτών-ξενιστών τους. Γίνεται συζήτηση για το *N. austeritella* ως ένα δυνητικά αποτελεσματικό παράγοντα βιολογικής αντιμετώπισης σε προγράμματα Ολοκληρωμένης Αντιμετώπισης του *P. farcta*.

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