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Morphology and biology of the elm leaf-mining moth, *Bucculatrix ulmifoliae* Hering, 1931 (Lep.: Bucculatricidae) in Iran

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Abstract
A survey on the morphology and biology of the elm leaf-mining moth *Bucculatrix ulmifoliae* was conducted under natural conditions. The developmental periods of 1th +2th, 3th, 4th and 5th larval instars as well as pupa were calculated as 6.40±0.67, 1.76±0.12, 1.92±0.20, 3.51±0.37, 9.61±0.77 days, in a cage at field; and 6.40±0.66, 1.50±0.27, 1.78±0.47, 2.71±0.48, 7.64±0.33 days at the laboratory (25±2°C, RH 65±5 and 16L: 8D hours) respectively. The average life span of the moth was obtained as 23.04±1.02 days at the laboratory and 25.84±0.91 days at the field. Adults of the first generation appeared in early April. The fertile females laid their eggs underside of leaves near the midribs. Upon hatching, the first instar larva penetrates directly via base of the egg into the leaf tissue and creates the mine. *B. ulmifoliae* had three generations of which the second generation showed its tendency for oviposition. Third generation was observed from the first half of August, and overwintering started at the first half of September. The first and second instars mined the leaf and fed inside, while the other instars fed externally on the underside. *Bucculatrix ulmifoliae* has three generations a year and overwintering as a pupa in a cocoon in the crevices of barks and fallen leaves on the ground.
Keywords: *Bucculatrix ulmifoliae*, *Ulmus*, morphology, biology, Elm

ریخت‌شناسی و زیست‌شناسی شب‌پره برگ‌خوار نارون، *Bucculatrix ulmifoliae* Hering, 1931 (Lep.: Bucculatricidae) در ایران

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چکیده
این تحقیق درباره شکل‌شناسی و زیست‌شناسی پروانه *Bucculatrix ulmifoliae* در شرایط طبیعی و آزمایشگاه انجام شد. نتایج نشان می‌دهد که *B. ulmifoliae* دارای سه نسل در سال است و به حالت شفیرگی در داخل شکاف تنه درختان و برگ‌های خزان شده روی زمین زمستان‌گذرانی می‌کند. دوره‌های رشد و نمو اول+ دوم، سوم، چهارم و پنجم لاروی و همچنین شفیرگی به ترتیب: ۰.٦۷ ± ٦.٤۰، ۰.۱۲ ± ۱.۷٦، ۰.۲۰ ± ۱.۹۲، ۰.۳۷ ± ۳.۵۱، ۰.۷۷ ± ۹.٦۱ روز، داخل قفس در مزرعه؛ و ۰.٦٦ ± ٦.٤۰، ۰.۲۷ ± ۱.۵۰، ۰.٤۷ ± ۱.۷۸، ۰.٤۸ ± ۲.۷۱، ۰.۳۳ ± ۷.٦٤ روز در آزمایشگاه (۲۵ ± ۲ درجه سانتی‌گراد، رطوبت نسبی ۵ ± ٦۵درصد و دوره نوری ۱٦ ساعت روشنایی و ۸ ساعت تاریکی) بود. متوسط طول عمر پروانه به ترتیب ۱.۰۲ ± ۲۳.۰٤ روز در آزمایشگاه و ۰.۹۱ ± ۲۵.۸٤ روز در طبیعت بود. افراد بالغ نسل اول در اوایل ماه اردیبهشت ظاهر شدند. ماده‌های بارور تخم خود را در پشت برگ و رگبرگ‌های میانی قرار می‌دهند. لاروهای جوان به طور مستقیم از قسمت پایه تخم به بافت برگ نفوذ می‌کنند و دالان ایجاد می‌کنند. پروانه *B. ulmifoliae* دارای سه نسل است. نسل سوم از نیمه اول ماه مرداد مشاهده شد و در نیمه اول ماه مهر به حالت شفیرگی در شکاف تنه درختان و یا زیر برگ‌های روی زمین به خواب زمستانی می‌روند.
واژگان کلیدی: *Bucculatrix ulmifoliae*، *Ulmus*، morphology، biology، Elm

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Introduction

Bucculatrix ulmifoliae Hering, 1931 is one of the 220 known world species of *Bucculatrix* Zeller, 1839 which is widespread in all parts of Europe (Langmaid *et al*., 2007). This species was first reported from Iran (Maleki *et al*., 2011) where it has recently become an important pest of elm trees, *Ulmus campestris* L. (Ulmaceae) causing extensive damages to the trees in the city of Tehran where it is feared that it will turn in to a key pest in the future. Till now, five species of *Bucculatrix* have been reported from Iran: *B. ulmella* Zeller which is reported on elm tree in Tehran region (Abai, 1997), *B. pomifoliella* Clemens, which is collected in Khorasan region on Apple tree (Shahrokhi, 1986), and the three species *B. iranica* Deschka, *B. endospiralis* Deschka and *B. pectinella* Deschka which are collected from the south of Iran and introduced as new species (Deschka, 1981).

The life history of the pest on elm trees is not studied in Iran. Biology of several *Bucculatrix* species such as *B. ivella* Busck, *B. canadensisella* Chambers and *B. ainsliella* Murtfeldt were known (Palmer & Diatloff, 1987; Slingerland & Crosby, 1915; Solomon *et al*., 1987). The aim of this study is investigate the biology of this species under natural conditions and at laboratory in Iran.

Materials and Methods

Adult morphology

Dissection and slide-mounting methods for genitalia and wings were based on those introduced by Clarke (1941) and Robison (1976). The abdomen was cut off and boiled for 2 or 3 minutes in 10% KOH. Preparations were stained with Chlorazol black and mounted in Euparal.

Biology

The biology of *B. ulmifoliae* was done in two different ways. In the first method, we selected 10 elm trees and inspected twice a day. A total of 40 infested leaves were cut off from the trees and transferred to leaf cages once the imprints of mines were observed on the leaf surfaces. The growth rate of each larva was recorded by daily visits. In the second method, the larvae were removed from the leaves as soon as they emerged from the mines at the laboratory and kept separately in the plates containing wet cottons at room temperature of 25±2°C and humidity of 65±5 percent with 16 and 8 hours lighting and darkness. The average duration of the insect developmental time for various larval instars and pupa in addition to the life cycle and sex ratio were calculated. Width of the head capsule of 120 larvae, which had emerged at the laboratory, was measured according to

Friend (1927). Ten specimens of each larval instar were selected to measure the average length of the body. The adults were sexed to determine the sex ratio. To determine the sex ratio of insects, their genitalia were removed and the number of males and females counted. The larval mortality was measured and analyzed using Excel software 2010.

Results and Discussion

Adult morphology

Wingspan of the examined adult specimens was 6.0–7.0 mm on the average (Fig. 1.A); all the wings characteristics were the same as the original description (Langmaid *et al*., 2007) except the forewing venations (Fig. 1.B) which in both fore- and hindwing reduced. In the forewing: Sc and Rs, and M_1 and M_2 merged together, respectively; R_3 has a common stalk with R_4; Cu veins absent. Posterior wing with only one Radial vein (R_5) and veins M_1 and M_2 merged with each other (Fig.1.B).

In the male genitalia, the two lobes of uncus somehow wider than that reported by Langmaid *et al*., (2007); valvae not exactly symmetrical; curvature of the phallus below the tip, more than 90° and two nipple-like projections of its posterior shaft were less conspicuous (Fig.1.C). The female genitalia is identical to that of Langmaid *et al*., (2007) (Fig. 1.D).

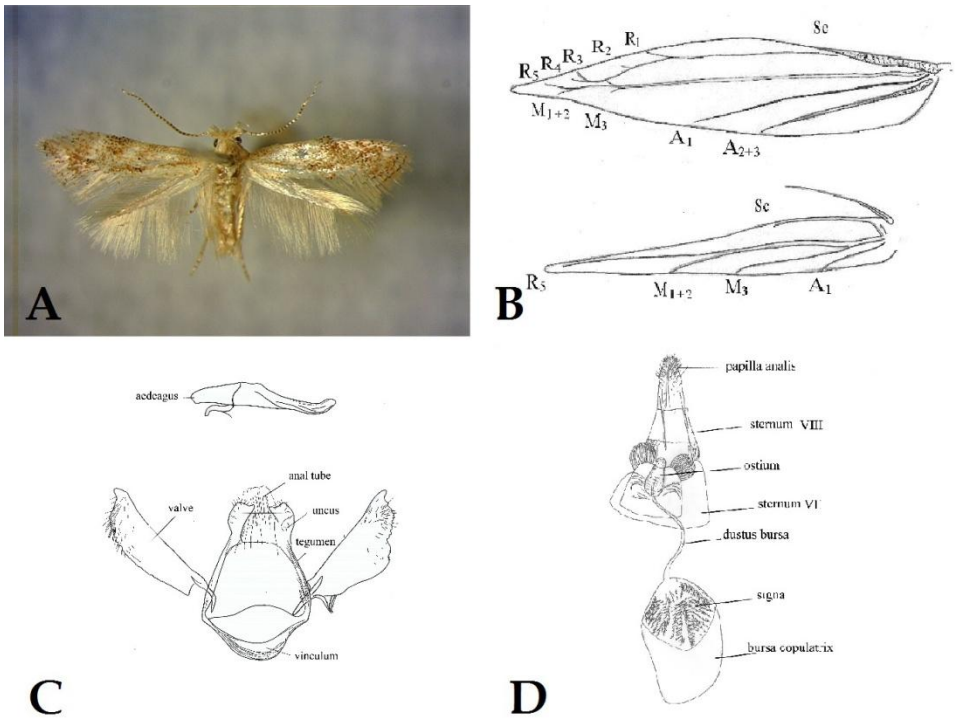

Fig. 1. A: *Bucculatrix ulmifoliae* adult (original), B: Front wing (above) and hind wing (down), C: Male genitalia. Magnification X100 (original), D: Female genitalia. Magnification X100 (original)

Eggs

Eggs oval, with obscure white color. Length of the ovum is 0.26 mm which is close to the size of the ovum in *B. canadensisella* (0.25 mm) (Friend, 1927) and longer than that of *B. parthenica* Bradley (0.14–0.22 mm) (McClay *et al.*, 1990). Width of the ovum is 0.15 mm. The adult females laid their eggs underside of leaves next to the mid vein.

Larvae

All the larval instars were pale yellow in color but they were bright green during feeding time (Fig. 2.A). The shape of those inside the mines (Fig. 2.B) was cylindrical with completely tangible leaf tissues in their digestive tracts. The body covered with scattered dark hairs which varied in different larval stages. Head, brown with five simple eyes positioned laterally. The average lengths of the first and second larval instars inside the mine were 0.36 mm; the third, fourth and last instars were 2.0–2.2 mm, 3.0–3.4 mm and 5.0–5.5 mm, respectively.

Pupae

Pupation occurs in a rod-shaped or fusiform cocoon; about 3.8–4.0 mm in length and 1.0 mm in width, bearing eight to nine sharp longitudinal ridges. The cocoon is mostly constructed on the undersurface of the leaf and rarely on its upper surface. Cocoon in all of the examined material (more than 200 specimens) was whitish and somewhat obscure (Fig. 2.C), although Langmaid *et al*. (2007) mentioned it as being dark gray.

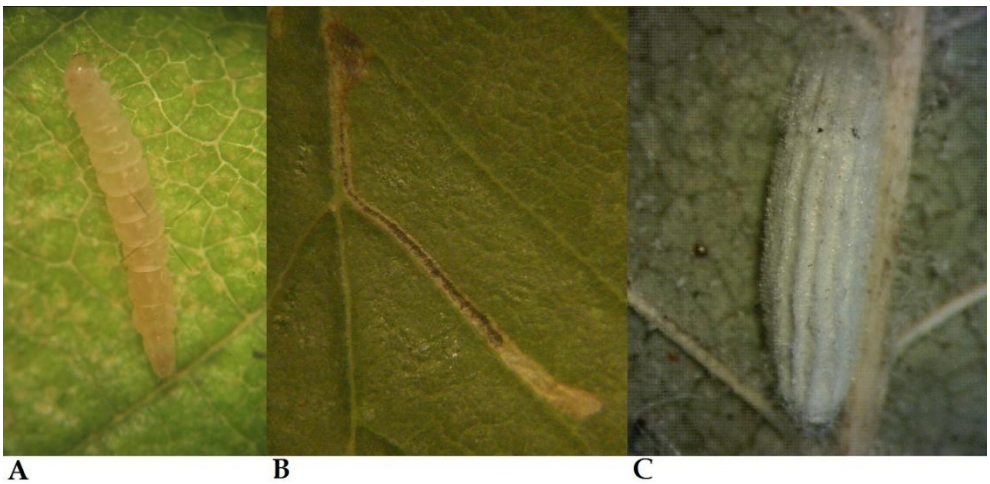

Fig. 2. A: *Bucculatrix ulmifoliae* larva (original), B: *Bucculatrix ulmifoliae* larval mine (original), C: *Bucculatrix ulmifoliae* pupae (original).

Life History

Bucculatrix ulmifoliae overwintered as pupa in a protective cocoon in the cervices of barks and or fell down leaves near the host trees. Adults of the first generation emerged in late April in the spring. The activity of the first generation was at the beginning of May to the mid-June, determined by yellow traps which placed on the barks. The second generation occurred in the early July. Duration of the second generation was shorter due to hot weather. This generation was observed in early July to the first half of August and overwintering started with the autumn season.

Mating and oviposition occurred in few hours after emerging of the adults in the field. The eggs were laid singly adjacent to the mid-vein and usually at the angle with a major vein underside of the elm leaves. The rest of the larval mines appeared within 7–11 days. The first and second larval instars fed and molted inside the mines. The third larval instars emerged on the leaf surface where it molted again after a day and made a hole at the end of the mine. According to the present study, *B. ulmifoliae* and *B. ivella* have five larval instars (Palmer & Diatloff, 1987) and their life cycles (from the first larval instar to the end of pupal stage) in the field condition include four generations. Moreover, the duration of larval instars and pupa including (1^{th} +2^{th} in total), 2^{th}, 3^{th}, 4^{th} and 5^{th} were 6.40±0.67, 1.76± 0.12, 1.92 ± 0.20, 3.51 ± 0.37, 9.61±0.77 days, respectively in the cage at field (Table 1); and 6.40±0.66, 1.50±0.27, 1.78±0.47, 2.71±0.48, 7.64±0.33 days, respectively at the laboratory (25±2°C, RH 65±5 and 16L: 8D hours) (Table 2). The average life span of a moth was calculated as 23.04±1.02 days at the laboratory and 25.84±0.91 days at the field.

In *B. pomifoliella*, the first molting on the outside of mine occurs after four days and longevity of the fifth larval instar lasts a week (Slingerland & Crosby, 1915). Molting process of larva in *B. ulmifoliae* on the leaf surface occurred after 1.7±0.6 days and longevity of the fifth larval instar was 3 1/2 days. In *B. pomifoliealla*, the life span of the larvae in the mine was one week (Slingerland & Crosby, 1915), while in *B. canadensisella* it was 13–50 days (Friend, 1927). Based on this study, the duration of 1^{st} and 2^{nd} larval instars (i.e., time of presence at the mine) was 6.4 days which is almost similar to that of *B. pomifoliealla*. The lifespan of *B. ulmifoliae* was 4–6 weeks which is similar to the lifespan of *B. canadensisella* (Friend, 1927). *Bucculatrix ulmifoliae* has three generations per year, while it is about 25 days in *B. parthenica* (McClay et al., 1990). According to Solomon *et al.* (1987) *B. ainsliella* has two generations per year which is the same as *B. pomifoliella* (Slingerland & Crosby, 1915).

The average lifespan of the males and females after their release and preservation was 7–13 and 8–11 days at the laboratory, respectively. The highest mortality at pre-adult stage due to environmental factors such as low humidity was calculated 48% in 1^{st} and 2^{nd} larval instar.

Table 1. The average duration of larval and pupal stages of *B. ulmifoliae* in natural conditions

Duration of insect development time	**average (Day) ± SE**
Average duration of larval in total from first to second	6.40 ± 0.67
Average duration of larval in thirdinstar	1.76 ± 0.12
Average duration of the firstmolting outside the mine	1.35 ± 0.14
Average duration of larval in fourth instars	1.92 ± 0.20
Average duration of the second molting outside the mine	1.26 ± 0.15
Average duration of larval in fifth instars	3.51 ±0.37
Average duration of pupal	9.61 ± 0.77
Insect development time except embryonic period	25.84 ± 0.91

Feeding Behavior

After eggs hatching, larvae bore directly into the leaf through the attached surface of the egg and begin mining the mesophyll. The thread-shaped mine was first lined next to the mid-vein. After roaming a small part in this area, the larval mines formed a curvature and moved to a triangle between mid-vein and major vein of the leaf, usually forming a small blotch where they have doubled back on itself; later extends in a straight line, usually along a vein, before turning away at an angle, with broken linear black trace in the canal with a total length of 15.0–18.0 mm.

Table 2. The average duration of larval and pupal stages of *B. ulmifoliae* at the laboratory

Duration of insect development time	**average (Day) ± SE**
Average duration of larval in total from first to second	6.400 ± 0.66
Average duration of larval in thirdinstar	1.50 ± 0.27
Average duration of the firstmolting outside the mine	1.33 ± 0.12
Average duration of larval in fourth instars	1.78 ± 0.47
Average duration of the second molting outside the mine	1.12 ± 0.11
Average duration of larval in fifth instars	2.71 ± 0.48
Average duration of pupal	7.64 ± 0.33
Insect development time except embryonic period	23.04 ± 1.02

In the angle of a mid-vein and major vein, the mines filled with black brasses. As stated by Langmaid *et al*., (2007), the first larval instar is a leaf miner in an 11.0–15.0 mm mine length. The exit-hole is positioned at the underside of the leaf, and the larva feeds externally on the underside of the leaves, creating small feeding windows. Moreover, the mines were wider at the beginning and then narrowed and 3–4 larvae were observed in some of these mines. The mines are more visible from the upper surface of the leaf. The

larva was present in the mines between 5 and 8 days. One or two larval head capsules with different diameters were observed in most of the mines. One of these capsules was in the middle of the mine and the other one located at the end of it. Therefore, the first and second instars mined the leaf and fed inside. The remaining instars fed externally on the underside of the leaf. In *B. parthencia*, the first and second instars were in the mines while the other instars were living outside the mine (McClay *et al*., 1990). Kobayashi *et al*. (2009) and Solomon *et al*. (1987) stated only the first larval instar of *B. hamaboella* and *B. ainsliella* as mines. Limited numbers of mines with only one head capsule were observed when the whole head capsule was missing as a result of molting. Diameter of the head capsules in the mine indicated that a number of larvae had left the larval mine without passing the second stage of molting. *Bucculatrix parthenica* infests *Parthenium hysterophorus* L. while its first and second larval instars are miners and the next generation feeds on parenchyma of the leaf (McClay *et al*., 1990). The species *B. pomifoliella*, which incurs damages to the apple trees, its first instars are miners of the leaves (Slingerl and & Crosby, 1915).

The difference in the whole lifespan may be related to the different hosts. After exiting the mine, the cylindrical shape of the first and second larval instars will turn flat, and they will feed from parenchyma of the leaf. The larvae frequented under the leaf and were rarely seen on the upper surface. This fact can be observed in the species that are considerably active in other plant species such as trees and shrubs. For example, in *B. canadensisella* which attacks Birch tree, its larvae feed on the underside of the leaf (Friend, 1927). In *B. ivella* which is a pest of *Baccharis* sp., the larvae were observed on both sides of the leaf; with more larvae occur underside (Palmer & Diatloff, 1987). The larvae become vulnerable when they exit the mines due to dehumidification or delay in the exchange of the fed leaves leading to heavy mortality among them. In addition, the larvae experienced two molting stages once they exited the mine. Two to three days after feeding, the larvae get their body curved as U- shaped under a thin covering or white cocoon being made of silk spun by their labial glands. The molting cocoon on the leaf has a flat form that is usually found along a midrib. The larva is motionless without feeding during the molting (or skinning up) stage. Molting lasts between one day and two days at most. In *B. hamaboella*, the two final larval instar stages complete within the framework of a molting operation, however, in *B. ulmifoliae*, each molting stage occurs in a separate and single cocoon.

In *B. hamaboella*, each larval age changing takes one day or two days (Kobayashi *et a*l., 2009) similar to *B. ulmifoliae*. Pulling out the larvae from the mines is resulted in their death within 1–2 hours. Larvae exited through the hole near the central part of the cocoon. The length of molting cocoon was usually half the length of larva, depending on the size of larva. It was also observed that the larvae exit the cocoon from the head part. When larva comes out of the cocoon, the remains of the skin change can be observed at the

bottom of cocoon, and diameter of the remained head capsule can be easily measured as has been also reported by Friend (1927). The fourth larval instars showed tendency towards the underside of the leaf, shortly after exiting the cocoon. In all their ages, larvae are feeding from underside of the leaf as the older larvae spine longer cords. The fourth larval instar entered molting stage at most four days of feeding; this stage lasted less than two days. At this stage, the size of these silky molting webs was measured to be approximately 2.5 mm. Therefore, the larvae have two molting stages outside the mine.

Fifth larval instar development duration is measured to be 5 1/2 days on average. It seems that the fifth larval instar can cause more damages to the leaf. During the day, moths live on the leaves and branches of the trees, and their activities intensify during the night.

Sex ratio

The sex ratio of both males and females in the field condition were calculated as 42% and 58%, respectively. Various larval instars were specified according to the diameter of their head capsules. The diameter of the head capsules remained from molted larvae inside the mines was obtained as 0.009, 0.010 and 0.015 mm. Those of remained from molted larvae on the leaf surface was 0.015, 0.021 and 0.026 mm, respectively (Table 3).

The second larval instar was inside the mine while the third one was on the outside. The average diameter of the head in the first, second, third, fourth and fifth larval instars were 0.0095, 0.0135, 0.0193, 0.0232 and 0.0277 mm, respectively (Table 3). These findings indicate that the second larval instar tends to move to the leaf surface after molting. Therefore, a small number of the latter larvae molt on the outside of the mine and then enter the third stage.

Table 3. Measuring the diameter of molt larval head capsule left in the cocoon.

Larvae instars	diameter of head capsules (mm)
Stage 2	0. 01
Stage 3	0. 015
Stage 4	0. 021
Stage 5	0. 026

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References

Abai, M. (1997) The report of *Bucculatrix* on Elm tree in Iran, Applied Entomology and Phytopatology 64(1/2), 25.

Clarke, J. F. G. (1941) The preparation of slides of the genitalia of Lepidoptera Bulletin of the Brooklyn Entomological Society 36,149–161.

Deschka, G. (1981) Blattminieren de Lepidopterenaus dem Nahen und Mittleren Osten. VI. Teil, Zeitschrift de Arbeitsgemeinschaft Östereichischer Entomologen 33, 33–41.

Friend, R. B. (1927) The biology of the birch leaf skeletonizer, Bucculatrix canadensisella, Chambers. [New Haven]: Connecticut Agricultural Experiment Station 288, 393–486.

Kobayashi, S., Hirowatari, T., Murase M. K. & Uroko, H. (2009) A new stem-borer of the genus Bucculatrix (Lepidoptera: Bucculatricidae) from Japan, with description of the life history. Entomological Science 12, 84–90.

Langmaid, J. R., Porter, J. & Collins, G. A. (2007) Bucculatrixulmifoliae Hering, 1931 (Lep.: Bucculatricidae) residentin England. Entomologist's record and Journal of Variation 119(9), 195–201.

Maleki, S. H. (2012) A study of biology, identification of natural enemies and population dynamic on natural parasitism of Bucculatrix ulmifoliae (Lep.: Bucculatricidae) in Tehran parks. M.Sc. Thesis.123pp. Islamic Azad University, Science, and Research Branch, Tehran, Iran.

Malekim, S., H., Baniameri, V., Alipanah, H. and Shojaee, M. (2011) First report of Bucculatrix ulmifoliae Hering (Lep.: Bucculatricidae) from Iran. Applied Entomology and Phytopathology 1(92), 157–160.

McClay, S., McFadyen, R.E. and Bradley, J.D. (1990) Biology of Bucculatrix parthenica Bradley sp.n.(Lepidoptera: Bucculatricidae) and its establishment in Australia as biological control agent for Parthenium hysterophorus (Asteraceae). Entomological Research 80, 427–432.

Palmer, W.A. and Diatloff, G. (1987) Host specificity and biology of Bucculatrix ivella Busck, A potential biological control agent for Baccharishalimifolia L. in Australia. Journal of Lepidopterists society 41(1), 23–28.

Robinson, G. S. (1976) The preparation of slides of the genitalia of Lepidoptera with special reference to the Microlepidoptera. Entomologists Gazette 27, 127–132.

Shahrokhi, M.B., Zare, A. and Fooladi. R. (1986) Bucculatrix pomifoliella (Lep.: Lyonetidae). Proceeding the 8th Iranian Plant Protection Congress, University of Technology, Esfahan 12.

Slingerlan, M. V. and Crosby, C. R. (1915) Manual of fruit insects. 503 pp. Macmillan New York.

Solomon, J. D., McCracken, F. I., Anderson, R. L., Lewis Jr., R., Oliveria, F. L., Filer, T. H. and Barry, P. J. (1987) Oak Pests: A Guide to Major Insects, Diseases, Air Pollution, and Chemical Injury. Protection Report R8-PR7. USDA Forest Service, Southern Forest Experiment Station New Orleans. 69 pp.