بيولوجيا وسلوكية السوسة *Microlarinus lareynii* Jacq. العدو الحيوي لعشب ضرس العجوز *Tribulus terrestris* L.

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الملخص

تمت دراسة بيولوجيا وسلوكية السوسة *Microlarinus lareynii* Jacq. العدو الحيوي لعشب ضرس العجوز *Tribulus terrestris* L. في مركز دراسات وبحوث المكافحة الحيوية، كلية الزراعة، جامعة دمشق خلال 2013–2016. وضعت الإناث البيوض في حفر في قمة الثمرة في فص واحد ووجد أن 50% من الثمار احتوت بيضة واحدة فقط. بلغ طول البيضة 0.51 –0.72 (0.58) مم، وعرضها 0.45– 0.56 (0.49). استمرت فترة حضانة البيض في المخبر من 3–5 (3.56) يوم. كانت اليرقة في العمر الأخير قوية وممتلئة ومقوسة، وبلغ طول جسمها 3.29–4.95 (4.31) مم. إكتمل الطور اليرقي في 15–25 (20.32) يوم. تبدأ اليرقات الفاقسة بالتغذي على أنسجة الثمرة وتحفر داخل الفص الأول ولاحقاً داخل الفص الثاني. يحدث التعذر داخل الثمرة المصابة ويدوم طور العذراء 3–6 (4) يوم. بلغ طول الحشرة الكاملة 3.12–4.55 (3.86) مم. يحمل النبات من 105–320 (128.16) ثمرة كان منها 45–116 (76.12) ثمرة مصابة بنسبة إصابة 34.43 – 43.36 (39.3) %. تقضي الحشرة فترة البيات الشتوي ابتداءً من تشرين الثاني على شكل حشرة كاملة بين البقايا النباتية تحت النبات العائل.

كلمات مفتاحية: بيولوجيا، سلوكية، *Microlarinus lareynii* Jacq.، ضرس العجوز *Tribulus terrestris* L.

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Biology and Behavior of Microlarinus lareynii Jacq. a Curculionid bioagent of Tribulus terrestris L.

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Abstract

Biology and behavior of a Curculionid bioagent, *Microlarinus lareynii* Jacq. attacking the cosmopolitan weed, puncturevine, *Tribulus terrestris* L. was carried out at Biological Control Studies and Research Centre, Faculty of Agriculture, Damascus University during 2013-2016. Female oviposited exclusively in top site of the fruits, in holes in one chamber, almost 50 % of the fruits were infested by one egg. The length of egg was 0.51 - 0.72 (av. 0.58) mm and width 0.45 - 0.56 (av. 0.49) mm. Incubation period in laboratory was 3-5 (av. 3.56) days. Last larval stage was stout, fleshy and curved with body length ranged from 3.29 to 4.95 mm with an average of 4.31 mm. The entire larval development completed within 15-25 (av. 20.32) days, the hatched larva feed on the fruit tissues and tunneled it and penetrate one or two chambers of the fruit. Pupation occurred inside infested fruits and this stage lasted for 3-6 (av. 4) days. The weevil length was 3.12 - 4.55 (av. 3.86) mm. The plant carried 105 - 320 (av. 128.16) fruits and the number of infested fruits by the weevil, was 45- 116 av. (76.12) fruits with an infestation percentage 34.43 to 43.36 (av. 39.3) %. The fully grown adults hibernate in November in debris under the plant canopy.

Key words: Biology, behavior, *Microlarinus lareynii* Jacq., puncturevine, *Tribulus terrestris* L.

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Introduction

The cosmopolitan plant, puncturevine, *Tribulus terrestris* L. belongs to Zygophyllaceae also known as bullhead or goat head is a native to Mediterranean region and North Africa (Andrés and Angelet 1963). It is a prostrate to decumbent, annual herb that bears very distinguishable small yellow flowers that transformed to prickly spiny fruits. First report of this weed in Syria was by Mouterde, 1983 and personal survey of T. terrestris reported the presence of the weed in almost all cities of Syria, and accompany all summer crops, with higher densities in uncultivated, orchards and vegetable fields. The plants reproduced only by seeds (Parsons and Cuthbertson 2001). The fruit consists of 5 wedge-shaped schizocarps (chambers), each with 1 or 2 pairs of large spines. There are usually 3 (av. 1-5) seeds per chamber (Scott and Morrison, 1996). Under optimum conditions a single plant can produce up to 400 fruits m^2 (Squires, 1969). Dispersal occurs via tyres, footwear, animals and movement of soil (Johnson, 1932; Goeden and Andrés, 1999). The seeds germinate rapidly after late spring and summer rains in warm conditions (24- 27°C). Several germinations can occur in one season. The seeds can remain viable for many years if buried in the soil, and thus after successive generations a large reservoir of seeds can accumulate (Squires 1979; Parsons and Cuthbertson 2001).

This species is known to cause photosensitization and nitrate poisoning in livestock and it can extract soil moisture from great depths and compete severely with crops in very dry conditions (Holms, 1977). The alkaloids in T. terrestris cause a locomotor disorder disease of livestock in Australia (Bourke et al. 1992; Bourke 2006). It is also associated with a photosensitisation disease called geeldikkop in South Africa (Kellerman and Coetzer 1984) with similar symptoms reported from Australia (Gastonbury et al. 1984).

It grows best where there has been soil disturbance, low competition from other plants, high temperatures and soil moisture with low atmospheric humidity. Human activity often provides these conditions hence the weed is regularly found associated with human activities in dry regions. Consequently the plant can be a nuisance for tourists, especially in camping grounds and for tyres.

Contamination of dried fruit by spiny burrs has been a problem in the Australian industry (Johnstone, 1990) and is managed by crop hygiene, cultural and herbicide methods (Mac Gregor, 1990). The only used way

to control this weed in Syria is the mechanical methods, using hands picking or simple tools, but its impractical because of the strong taproot and spiny fruits and the wide area of spread, therefore the biological control offers a promising measure.

The seed feeding weevil *Microlarinus lareynii* (Jacquelin de Val) was reported as the most promising candidate for use as biological control agent at many places around the glob. Field and laboratory studies conducted in France, Italy and California during 1959-1961 demonstrated that the reproduction succeeded only on puncturevine (Andrés and Angelet, 1963). Weevil adults were initially imported from Italy and released directly in the field in Arizona, California, Colorado, Nevada, Utah and Washington in July and August, 1961 (Huffaker et al. 1961, Andrés and Angelet 1963); establishment occurred in Arizona, California and Nevada (Maddox, 1976). The weevils established readily in California and spread rapidly and widely, aided by extensive transfers of field-collected adults (Goeden and Ricker, 1967, Maddox, 1976). Kirkland and Goeden (1978), showed that the weevil attack caused a 60% reduction of flower production on surviving plants. Huffaker et al. (1983) reported that 15 years after introduction of the weevils, puncturevine coverage and seed production declined in more than 80% of 1,200 field plots monitored in California. They attributed this decline to the actions of the weevils. The biological control of puncturevine in California generally is considered a partial success or substantial success under field conditions (Maddox and Andrés, 1979, Goeden and Andrés, 1999).

Therefore, study on biology and behaviour of M. lareynii was carried out during 2013-2016 at the Biological Control Studies and Research Centre, Faculty of Agriculture, Damascus University, Damascus, Syria.

Material and Methods

Stock cultures

Puncturevine plants

Seeds of puncturevine were collected from the farm of Agriculture Faculty, Damascus, Syria, during 2013- 2014 and kept at the Seed Bank of weed seeds at the Biological Control Studies and Research Centre. Seed were grown in pots (15 cm diameter) on stands in the Net House. Ten whole fruits were sown in each pot, and only 5 plants allowed to grow. Each five pots were kept in separated stand covered with special net designed for this purpose. Another stock culture was planted in soil in separate room in the net house.

The weevil, *M. lareynii* Culture.

The stock of the weevil culture was initiated by collecting infested puncturevine fruits from fields during September 2013. According to our experience the infested fruits, during this month, contained the pupal stage or matured adults. The collected weevils (200 individuals) were released on healthy plant of *T. terrestris* grown in net house. At the beginning of the next summer the seeds of T. terrestris were germinated again and started to grow at the beginning of July, the adults of *M. lareynii* was first seen in the net-house during September, 2015 and kept under continuous observations.

Egg incubation:

Eggs were removed from the infested fruits with the help of forcipes and small brush under the microscope. Twenty five eggs were incubated on moist filter paper in a covered petri dish in a chamber (Platner et al., 1973).

Biometrical characters:

Biometrical characters of eggs, larval instars, pupa and adults were recorded with the help of ocular and stage micrometer fitted in microscope.

Grub and pupal stages: Newly infested fruits on plants grown in pots were kept under net cages and frequently observed. Fruits were opened to determine some characters of the larvae and they returned to the same fruit or another fruits. Characters of the first and last larval instar were recorded and also observations on length, breadth and morphological features of pupa were recorded.

Adult behavior: Ten couples of adults were collected during mating and kept separately inside plastic container made for insect rearing in the net-house. Pots with already grown *T. terrestris* were put inside the container. Mating behavior and egg laying were observed and recorded. Number of

attacked fruits by the adults were noticed and infestation percentage calculated. Adult characters and measurements were recorded.

Field Infestation.

Ten locations were located in Abu Jarash (main farm, gardens and the net house of the Biological Control Studies and Research center). At every location three whole plants were cut carefully at soil level and all fallen fruits were gathered and put in nylon bags and moved to laboratory, to calculated the infestation.

Results

Biology of *M. lareynii*

Egg. Females oviposited exclusively in top site of the newly-set fruits of T. terrestris. The eggs laid in holes made by the female with rostrum in one chamber of the fruits and could be located as small or tiny brownish spots which turned later into black. Kirkland and Goeden, (1977) mentioned that the eggs were laid singly in the fruits. After dissection of 100, immature, field-collected fruits those showed symptoms of initial infestation, found that 50 % of the fruits were infested by one egg, 20 % infested with three eggs, 20 % infested with two eggs in separated chamber however the remaining fruits (10%) are resulted from feeding by the insects or other reasons (no eggs were found in pits). Goeden and Ricker (1970) reported as many as 10 eggs have been observed on a single fruit with 53% of the inspected fruits.

However, the egg laying did not interrupt the growth of the fruits. The site of oviposition showed blackening of the damaged tissues around the laid egg.

The freshly laid eggs were transparent and oval in shape and pale cream in color. Length ranged from 0.51 - 0.72 with an average 0.58±0.06 mm and width ranged from 0.45 - 0.56 with an average 0.49±0.03 mm (Table 1).

Table 1. Dimensions of eggs of *M. lareynii*

Length (mm)		Width (mm)	
Range	Mean	Range	Mean
0.51 - 0.72	0.58±0.06	0.45 - 0.56	0.49±0.03

The incubation period in laboratory was 3 to 5 (av. 3.56 ± 0.71) days in September, 2014.

Grub: The neonate grubs were transparent and pale cream coloured. The length of the 1st instar ranged from 1.26 to 2.24 mm with an average of

1.63 ± 0.27 mm (Table 2). The width of the head capsule ranged from 0.15 to 0.29 mm with an average of 0.21 ± 0.04 mm.

Table 2. Body length and width of head capsule of the first and last grub instars.

Grub instars*	Body length (mm)		Head width (mm)	
	Range	Mean	Range	Mean
Ist	1.26 - 2.24	1.63 ± 0.27	0.15 - 0.29	0.21 ± 0.04
last	3.29 - 4.95	4.31 ± 0.47	0.55 - 0.89	0.68 ± 0.11

The last instar (before pupation), the larvae stopped feeding and movement, the body colour changed gradually from whitish to milky white at the end of the last instar. Grub was stout, fleshy and curved. The body length ranged from 3.29 to 4.95 mm with an average of 4.31 ± 0.47 mm. The width of the head capsule ranged from 0.55 to 0.89 mm with an average of 0.68 ± 0.11 mm (Table 2).

The entire larval development completed within 15 to 25 days with an average of 20.32 + 3.47 days. Goeden and Ricker (1970) reported that twenty larvae reared to maturity in forcibly infested fruits on caged, potted plants under insectary conditions completed their development in 13-16 days in California, USA, which was less than what we observed in this study.

Behaviour of the grubs, after hatching, the neonate grub immediately tunneled into the young fruits tissues, later tunneled the whole chamber and this feeding led to interrupt the seed development inside the infested chamber. In many cases the larvae later tunneled the neighbouring chamber and pupated inside it and in few cases we observed the larva penetrate the third chamber. Andres and Angelet (1963) found the larva generally destroy all seeds in a fruit chamber before moving on to the next. In addition to the direct damage by the larvae feeding Goeden and Ricker (1973) suggested that the feeding may provide portals of entry for secondary pathogens. Puncturevine fruit usually are comprised of 3-5 chambers, so a single larva was capable to destroy almost more than the half of the fruit. The 100 infested, mature fruit collected at Abu Jarash contained an average of 9.5 (range: 4—13) seeds, of which an average of 6.4 (range: 2-11) or 68% were destroyed by M. lareynii. Comparable data from Moreno by Kirkland and Goeden (1977) were 8 (range: 0-12) seed/fruit, with 5.8 (range: 3-12) or 64% destroyed. Andres and Angelet (1963) reported that 1-3 weevils may complete their development in 1

fruit, but unfortunately in this study it could not observe more than one larva in each fruit and this may be due to less population of this bioagent in the research site.

Pupa. The pupation commenced within the excavated fruits between 2 or 3 adjacent chambers. The pupa was in an open cell surrounded by loosely packed larval frass and was located in the center of the fruit. Pupae were creamy white or pale yellow. Body length was 3.29 to 4.94 (av. 4.02 ± 0.44) mm, Head width was 0.58 to 1.32 (av. 1.05 ± 0.14) mm (Table 3).

Table 3 .Body length and width of head capsule of the Pupa

Pupa	Body length (mm)		Head width (mm)	
	Range	Mean	Range	Mean
	3.29 - 4.94	4.02 ± 0.44	0.58 - 1.32	1.05 ± 0.14

The pupal stage lasted 3-6 (av. 4 ± 1.02) days for 25 individuals under laboratory conditions.

The duration from egg to adult stage takes 21-36 (av. 27.88) days. Which was quite longer than that observed by Goeden and Ricker (1970) as they mentioned the mentioned duration was 19-24 days in California, USA.

Adult. The males and females were separated during mating and distinguished depending on the 8th abdominal sternite as was described by Andres and Angelet (1963). The adults measurements varied greatly depending on the size of the infested fruits. The average length and thorax breadth of the males and females were 3.12 - 4.55 (av. 3.86 ± 0.37) and 0.9 - 1.76 (av. 1.18 ± 0.17) mm, respectively (Table 4). Male to female sex ratio was 1.08 : 1.

Table 4. Body length and width of breadth (at Thorax) of the Adult

Pupa	Body length (mm)		Breadth (mm)	
	Range	Mean	Range	Mean
	3.12 - 4.55	3.86 ± 0.37	0.9 - 1.76	1.18 ± 0.17

The adult emerged from the fruit through a circular exit hole. Adult started eating immediately on the leaves of the plants and fresh stem (undersides), later it could feed on all parts of the plants making many holes on leaves and stem and fruits. Feeding occurred during daylight hours. Feeding of the adult on the peduncles of the fruits may cause addition damage as it cause the buds, flowers, or fruit fall off. Also feeding on the fruits may cause abnormal fruit shape as one or more chambers stopped their growth.

It was observed that during night till early hours in the morning weevils are found on the soil under the plant canopy and during the day hours they spread all over the plant. In laboratory the first mating was observed to start in the second day after adult emergence from the fruits. Kirkland and Goeden, 1977 observed the 1st mating usually occurred 2-3 days after emergence. Fruits of the weed collected during November were inspected and fully grown adults were found to hibernate inside them and this was observed by Andres and Angelet (1963) and Kirkland and Goeden, 1977. The hibernated insects affected greatly by the farming practices especially plowing the ground.

It was difficult to determine the number of generations in this study, but Andres and Angelet (1963) reported that 1-3 generations occurred in Italy; 3 in France, conceivably, 4 annual generations may occur during the 6 months growing season of puncturevine in parts of southern California.

At the beginning of November the adults started to hide in plant litter beneath plants, and remain inactive (hibernated) till next July.

Weed characters and field infestation: The plant of T. terrestris cover a large area in the field. It has many branches 3-7 (av. 4.84 branches) with an average of branch length 64.84 cm. The weed was prolific with a fruit number ranged from 105 to 320 (av. 128.16 fruits).

Thirty plants at seven locations in Abu Jarash farm were inspected for infestation of the M. lareynii showed the following data (Table 5).

Table 5.Average of infestation of M. lareynii in Abu Jarash farm.

Branches No.	Branch length (cm)	Fruit No.		
		intact	infested	infestation (%)
3-7 av. (4.84)	10-120 av. (64.84)	60-204 av. (122.04)	45- 116 av. (6.12)	34.43- 43.36 av. (39.3)

The number of infested fruits by the weevil, was 45- 116 av. (76.12) fruits with an infestation percentage ranged from 34.43 to 43.36 with an average (39.3%). The infestation or feeding by the adult at early stage of the fruit growing may caused deformed shaped of fruits or small ones with less number of seed chambers. Kirkland and Goeden (1978), showed that the weevil attack caused a 60% reduction of seed production on surviving plants.

It was found at Abu Jarash Farm that the plant with the highest fruit number showed less infestation, as the plant with 105 fruits, 45 fruits

were infested (42.86 %) while the plant with 320 fruits, 116 fruits were infested (36.25 %) and this may due to lower population of the weevil. Huffaker et al. (1983) reported that 15 years after introduction of the weevils, puncturevine coverage and seed production declined in more than 80% of 1,200 field plots monitored in California.

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