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Biology of *Chamaesphecia schizoceriformis* (Lep.: Sesiidae), a biocontrol agent of *Euphorbia boissieriana* (Euphorbiales: Euphorbiaceae) in north west of Iran

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Abstract

The genus *Euphorbia* L. (Euphorbiales: Euphorbiaceae) typically have a poisonous white sap in all plant parts. Weedy spurge, *Euphorbia boissieriana* Prokh., is a toxic deep-rooted herbaceous and perennial weed that is native to the north west of Iran. The biology of spurge clear-wing moth *Chamaesphecia schizoceriformis* Kolenati as a biocontrol agent of *E. boissieriana* was studied at field and laboratory conditions (23 ± 2ºC, 60-70% RH, 14L: 10D h) during 2002-2004 in Orumieh region. A field and laboratory observation showed that spurge clear-wing moth was a univoltine species feeding on a single host plant, *E. boissieriana*. The flight period extended from mid June to the beginning of August. Females, after mating, laid their eggs singly on vegetative stems, mostly on the upper part of the older plants. Females laid an average of 205 ± 29 eggs and potential fecundity was 223 ± 27. The larvae of *C. schizoceriformis* hatched after 7.90 ± 0.23 days at mean daily temperature of 24.2 ºC. The young larvae penetrate into the stem a few centimetres above the ground and then move into the pith and down into the root. Based on the measurements of head capsules, this moth has seven larval instars and reaches the 5th or 6th instar before winter. In the spring, the larvae mined up to the stem base and prepared an emergence hole a few centimetres above the ground and then pupated within the stem. At mean daily temperature of 20.2 ºC, the pupal period was 13.43 ± 0.27 days. Study on parasitoids of *C. schizoceriformis* revealed that the larvae and pupa of this moth were parasitized by *Villa sesivora* Greathead & Karimpour (Dip.: Bombyliidae), *Bithia glirina* (Rondani) (Dip.: Tachinidae) and *Phaenolobus saltans* (Gravenhorst) (Hym.: Ichneumonidae) in Orumieh region.

Key words: biology, *Chamaesphecia schizoceriformis*, *Euphorbia boissieriana*, Orumieh, West Azerbaijan

چکیده

علف‌های هرز جنس *Euphorbia* L. (Euphorbiales: Euphorbiaceae) به‌طور مشخص دارای شیرابه‌ی سمی سفید رنگ در تمام بخش‌های گیاهی خود هستند. علف هرز *Euphorbia boissieriana* Prokh. گیاهی است علفی، سمی، دائمی و دارای ریشه‌ی عمیق که بومی شمال غرب ایران می‌باشد. زیست‌شناسی شب‌پره‌ی بال شفاف فرفیون، *Chamaesphecia schizoceriformis* Kolenati به عنوان عامل کنترل بیولوژیکی این علف هرز در طول سال‌های ۱۳۸۱ تا ۱۳۸۳ در شرایط صحرایی و آزمایشگاهی (دمای ۲ ± ۲۳ درجه‌ی سانتی‌گراد، رطوبت نسبی ۷۰–۶۰ درصد و دوره‌ی نوری ۱۴ ساعت روشنایی و ۱۰ ساعت تاریکی) در منطقه‌ی ارومیه مطالعه شد. بررسی‌های صحرایی و آزمایشگاهی نشان داد که این شب‌پره دارای یک نسل در سال بوده و میزبان گیاهی آن تنها به گونه‌ی *E. boissieriana* محدود می‌شود. ظهور حشرات کامل شب‌پره‌ی بال شفاف فرفیون از اواخر خرداد آغاز و تا اواسط مرداد ادامه یافت. حشرات ماده بعد از جفت‌گیری تخم‌های خود را به‌صورت انفرادی روی ساقه‌های رویشی در اندام‌های فوقانی گیاه میزبان چند ساله قرار دادند. میانگین تعداد تخم گذاشته شده توسط هر فرد ماده و توانایی تخم‌ریزی آن به‌ترتیب ۲۹ ± ۲۰۵ و ۲۷ ± ۲۲۳ عدد بود. تخم‌ها در میانگین دمای روزانه‌ی ۲۴/۲ درجه‌ی سانتی‌گراد در طول ۰/۲۳ ± ۷/۹۰ روز تفریخ شدند. لاروهای جوان با سوراخ کردن ساقه در چند سانتی‌متری بالای سطح خاک، به مغز ساقه نفوذ و به طرف ریشه حرکت می‌کنند. اندازه‌گیری عرض کپسول سر نشان داد که این شب‌پره دارای ۷ سن لاروی بوده و قبل از زمستان به سنین لاروی ۵ یا ۶ می‌رسد. در بهار سال بعد، لاروها از ریشه به طرف طوقه‌ی گیاه رفتند و در چند سانتی‌متری بالای سطح خاک یک حفره خروجی تعبیه کرده و در همانجا تبدیل به شفیره شدند. در میانگین

دمای روزانه ۲۰/۲ درجه‌ی سانتی‌گراد، دوره‌ی شفیرگی ۰/۲۷ ± ۱۳/۴۳ روز طول کشید. بررسی‌های منطقه‌ای مربوط به پارازیتوئیدهای این شب‌پره نشان داد که لاروها و شفیره‌های آن توسط مگس *Villa sesivora* Greathead & Karimpour از خانواده‌ی Bombyliidae، مگس *Bithia glirina* (Rondani) از خانواده‌ی Tachinidae و زنبور *Phaenolobus saltans* (Gravenhorst) از خانواده‌ی Ichneumonidae پارازیته می‌شوند.

واژگان کلیدی: زیست‌شناسی، *Chamaesphecia schizoceriformis*، *Euphorbia boissieriana*، ارومیه، آذربایجان غربی

Introduction

The genus *Euphorbia* L. (Euphorbiales: Euphorbiaceae) contains about 1600 species native to Africa, Asia, Europe, and North America (Gassmann & Schroeder, 1995). Euphorbiaceae typically have a poisonous white sap in all plant parts that irritates the eyes, mouth, and gastrointestinal tract and causes dermatitis upon contact in some people (Westbrooks & Preacher, 1986). This toxic latex appears to be for defensive purposes (Gassmann & Schroeder, 1995). Leafy spurge (*Euphorbia esula* L.) is a Eurasian perennial weed that was introduced into North America in the 19th century. It infests several million hectares of rangelands and riparian areas in the United States (Dunn, 1979). Annual direct and indirect economic losses due to leafy spurge infestation in Montana, South Dakota, North Dakota and Wyoming are estimated to exceed $120 million and could reach $144 million annually by 1995 (Leitch *et al.*, 1994). Since 1988, the United States Department of Agriculture, Plant Protection and Quarantine, Animal and Plant Health Inspection Service (USDA–APHIS–PPQ) has coordinated a classical biological control implementation program against leafy spurge in the United States. Through this program, two species of root-boring Sesiid moth *Chamaesphecia hungarica* (Tomala) and *C. tenthrediniformis* (Denis & Schiffermüller) have been imported and released in the United States (Hansen *et al.*, 1996). Seventy species belonging to the genus *Euphorbia* has been reported from Iran (Ghahreman & Attar, 1998), of which the weedy spurge, *Euphorbia boissieriana* Prokh., is native to West Azerbaijan. This plant is a toxic deep-rooted herbaceous and perennial weed.

Sesiidae is a cosmopolitan family of 1063 described species (Heppner & Duckworth, 1981). Morphologically it is a well-defined group of insects with transparent wings and bright coloured rings on the abdomen, mimicking wasps and bees (Fibiger & Kristensen, 1974). The genus *Chamaesphecia* Spuler, is restricted to the western and central Palaearctic region and comprises 82 species (Lastuvka, 1988; Spatenka *et al.*, 1999), however, Tosevski (1993) described another species from the eastern Palaearctic. No species of Sesiidae has been recorded on native North America spurge species (Eichlin & Duckworth, 1988). Lastuvka

(1988) recognized two subgenera within *Chamaesphecia* according to the morphology of the male genitalia and the host plant of the moth, the subgenus *Chamaesphecia sensu stricto* which is associated with *Euphorbia* species, and the subgenus *Scopulesphecia* which is associated with plant species of the families Laminaceae, Scrophulariaceae and Hypericaceae.

Twenty-three of the 82 Palaearctic *Chamaesphecia* species are associated with *Euphorbia* species. Most *Chamaesphecia* species for which host plants are known feed on only one plant species or a few very closely related plant species (Lastuvka, 1988). Ten *Chamaesphecia* species and nine host plant species in 88 sites in eastern and south-eastern Europe were surveyed and described by Tosevski *et al.* (1996). Pühringer & Kallies (2005) listed the name of *C. schizoceriformis* in the checklist of the Sesiidae of the world, but there is no adequate information on biology and ecology of this species and its closely related species. The current study was carried out in order to determine the biology, parasitoids, host range and impact of *C. schizoceriformis* – which was recently reported from Iran (Karrimpour *et al.*, 2005a) – on weedy spurge for employment of this moth in biological control programs against the species of *Euphorbia*.

Materials and methods

This study was carried out during 2002-2004 in Orumieh, Iran under field and laboratory conditions.

Field study: in order to determine the number of generation and emergence peak of spurge clear-wing moth, 20 bushes of *E. boissieriana* were each covered with a cage (50×60×50 cm), early in the April each year. Sides of each cage were covered with a cloth net. Bushes were selected randomly in Nazlo region, an area of 100 hectares, located at an altitude of 1293 m and 12 km west of Orumieh. The cages were visited at 2-3 days intervals and after first adult emergence they were checked daily. During the emergence of adults, date and numbers of emerged adults were recorded. At the peak of their emergence, weedy spurge bushes were carefully visited to find out oviposition sites. In order to determine host plant range of this moth, 10 bushes from each species of *Euphorbia seguieriana* Neck., *E. macroclada* Boiss., *E. denticulata* Lam. and *E. heteradena* Jaub. & Spach. were covered randomly with cages in the above-mentioned dimensions (each bush covered by one cage). The cages were visited at short intervals for checking the emergence of Sesiid adult moth.

Laboratory studies: in order to obtain the mean number of eggs per female, early emerging males were kept in small glass vials (2.5×6.5 cm) in a dark container at 11-13 ºC to

reduce their activity and preserve their reproductive capability. The females were put into transparent plastic cylinders (14×18 cm) and exposed to natural daylight. Females were exposed to males only when they were ready for mating, generally within 24 h of emergence. Two males were exposed to each female. Oviposition was obtained on 15 cm long cut shoots inserted in pots filled with sand and covered with a plastic cylinder (25×40 cm) in 12 replicate. Ovipositing females were fed with a few drops of 10% honey water solution. According to Tosevski *et al*. (1996), potential fecundity was measured by dissection of the females. In order to obtain parasitoids, clear-wing infested roots of *E. boissieriana* were collected from Nazlo region and maintained in the laboratory for the emergence of parasitoids. The laboratory temperature and relative humidity were 22-27 ºC and 60-70%, respectively. Infested roots of *E. boissieiana* were collected from fields in monthly intervals, as well as hatching larvae in the laboratory, to measure the head capsules of larvae in different stages. Collected roots were dissected and head capsules of larvae were measured according to Genc *et al*. (2003).

Results and Discussion

Bionomics

Field and laboratory studies revealed that weedy spurge clear-wing moth is a univoltine species. The adults of *C. schizoceriformis* emerged between mid June to early August in Orumieh region. The same results obtained by Spatenka *et al.* (1999) in Artwin, Turkey and Georgia. The mature larvae moved from the root to the lower part of the stem, where they made an exit hole, from which adults emerged, and pupated. During emergence, the chrysalid skin was dragged outside of the stem for ¾ of its length and held in place by anal hooks while the moth freed itself (fig. 1). Mating occurs in the morning within 24 hours of emergence. Under laboratory conditions, about 75% of mating was successful when a newly emerged female was placed with two males in a horizontal plastic cylinder (14×18 cm). It was important not to add the males until the movement of the female abdomen indicated she was calling and the containers had to be cleaned after copulation in order to remove any pheromone. Best oviposition was obtained by offering 15 cm long spurge stem stuck into moist perlite and covered with a plastic cylinder. Oviposition began shortly after mating with a photoperiod of 16 hours daylight and 22 ± 2º C. Average female longevity was 5.38 ± 0.18 days (range 5-6) and they laid an average of 205 ± 29 eggs (range 155-241) from a potential fecundity of 223 ± 27 eggs (range 173-252). In the field, the eggs were usually laid singly and

during the weedy spurge flowering period more than half of them laid on the bracts and a few on the stem. The eggs were laid both on the leaves (mostly on the upper surface) and on the stem before and after flowering. The average length and width of the eggs were 0.797 ± 0.003 and 0.549 ± 0.002 mm, respectively. The eggs were oval in shape and flattened with a network of slightly raised veins forming pentagonal and hexagonal shapes on the external surface (fig. 2). They were dark brown in colour when laid, and then they generally became light brown after the emergence of the larvae. The egg incubation period of *C. schizoceriformis* was 7.90 ± 0.23 days (range7-9) at mean daily temperature of 24.2 ºC. The neonate larvae penetrated into the shoot a few centimetres above the ground. The young larvae mined the stem and then moved into the pith and downed into the root. Third instar larvae generally started to penetrate the central part of the root. Most of the larval feeding was done in the root. Fully grown larvae made tunnels of 7-17 cm long in the root. These tunnels were tightly filled with larval excrement. Up to 12 larvae was observed in a single root. Measurements of head capsules indicated that this moth had probably seven larval instars (table 1) and reached the 5^{th} or 6^{th} instar before winter. In the spring, the larvae mined up to the stem base and prepared an emergence hole a few centimetres above the ground and then pupated within the stem. At mean daily temperature (20.2 ºC; range 18.3-22.7), the pupal period was 13.43 ± 0.27 days (range 12-15). The empty pupal case was left protruding from the stem after the emergence of adult. Seasonal activities of *C. schizoceriformis* under field conditions are presented in figures 4 and 5.

Host range in the field and greenhouse testing

The host-plant range of *C. schizoceriformis* was investigated by testing five plant species of Euphorbiaceae. This test showed that *C. schizoceriformis* fed only on weedy spurge, *E. boissieriana*. According to Spatenka *et al.* (1999), the host range of this moth in Artvin, Turkey and Georgia is also restricted to the same plant. In Europe, *Chamaesphecia* spp. attacking spurges are monophagous, with the exception of *C. tenthrediniformis*, which develops in two closely related species, *Euphorbia salicifolia* and *E. esula*. The host plant range of *C. empiformis* (Esper), *C. astatiformis* (Herrich-Schäffer), *C. hungarica*, *C. bibioniformis* (Esper), *C. myrsinites* Pinker and *C. palustris* Kautz are restricted to *Euphorbia cyparissias* L., *E. esula*, *E. lucida* Waldst. & Kit, *E. seguieriana* Necker, *E. myrsinites* L., and *E. palustris* L., respectively (Tosevski *et al.*, 1996).

Figure 1. The chrysalid skin of *C. schizoceriformis* (arrowed) dragged outside of the stem.

Figure 2. Eggs of *C. schizoceriformis* showing a network of slightly raised veins.

Table 1. Measurement of head capsule and length of larvae of *C. schizoceriformis* in each instar (Mean ± SE; N = 10).

Larval instar	Head capsule measurement (mm)	Length (mm)
First	0.219 ± 0.008 a	0.72 ± 0.08 a
Second	0.534 ± 0.007 b	7.34 ± 0.12 b
Third	0.874 ± 0.004 c	12.37 ± 0.24 c
Fourth	1.124 ± 0.004 d	16.48 ± 0.29 d
Fifth	1.437 ± 0.008 e	21.45 ± 0.16 e
Sixth	1.726 ± 0.005 f	26.86 ± 0.33 f
Seventh	2.182 ± 0.003 g	31.84 ± 0.41 g
LSD	0.0061	0.0462

The means within a column followed by different letters are significantly different ($P < 0.05$).

Figure 3. Last instar larva of *C. schizoceriformis.*

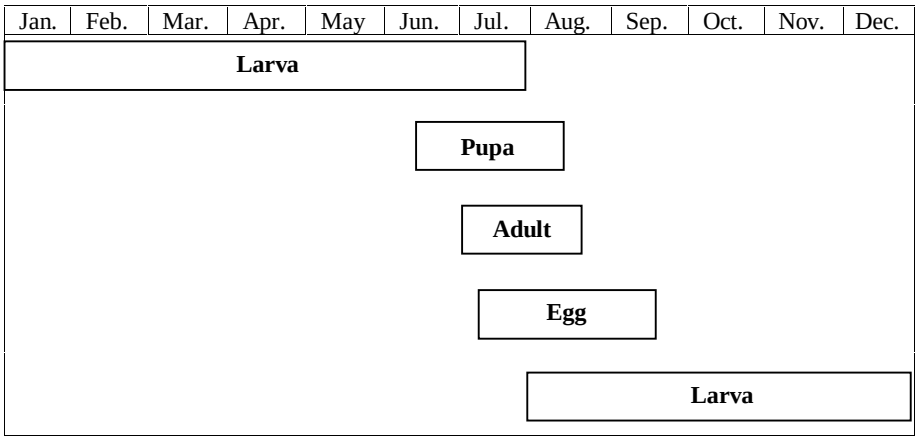

Figure 4. Diagrammatic representation of seasonal activities of *C. schizoceriformis* under field conditions.

Impact of *C. schizoceriformis* on weedy spurge

This study showed that weedy spurge was sensitive to root damage and consequently susceptible to *C. schizoceriformis* as the larvae feed on its root. Field observations showed that 100% of four and five years old bushes of *E. boissieriana* were attacked by the larvae of *C. schizoceriformis* and up to 27% of older bushes died in the area. Therefore, the direct effect of the root-boring moth on weedy spurge will be the reduction of the weed density. The

feeding behaviour of the larval stage can cause severe damage to the root cortex below the crown of the plant. Late instar larvae penetrate the central part of the roots. This feeding opens the root to invasion of soil born pathogens further reducing plant vigour. *C. schizoceriformis* plays an important role in the biological control of weedy spurge in West Azerbaijan. This moth was recommended as a candidate for biological control agent of *E. esula* in North America.

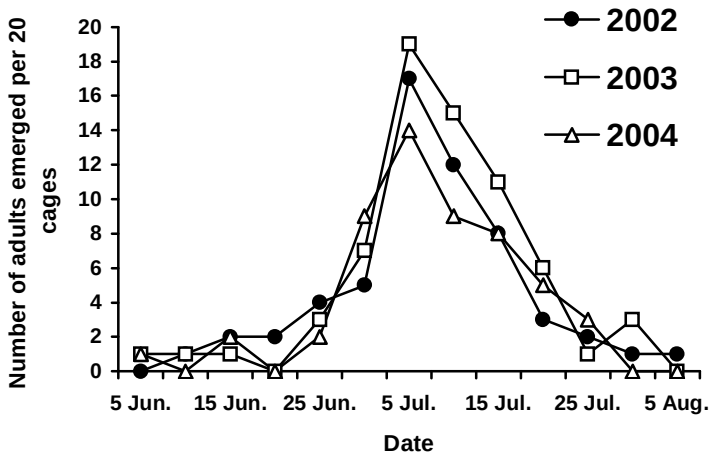

Figure 5. Seasonal changes of *C. schizoceriformis* adult flight in Orumieh region, North West of Iran during 2002-2004.

Parasitoids of *C. schizoceriformis*

Research on parasitoids of *C. schizoceriformis* in Orumieh region showed that this moth was parasitized by the following three species: 1. *Villa sesivora* Greathead & Karimpour (Dip.: Bombyliidae), which was recently described as a new species to science (Greathead & Karimpour, 2006), 2. *Bithia glirina* (Rondani) (Dip.: Tachinidae: Tachininae), which is a very rare species having one generation per year (Vanhara, 1999) and has been reported from southern and central Europe as a parasitoid of some species of *Chamaesphecia*, and 3. *Phaenolobus saltans* Gravenhorst (Hym.: Ichneumonidae: Acaentinae), which is distributed in Palaearctic region (Sedivy, 1955). The latter two parasitoids were recently reported from Iran (Karimpour *et al.*, 2005b, 2006).

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