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## The Life History and Biology of *Aphthona russica* sp. nov. (Coleoptera: Chrysomelidae: Alticinae), a Potential Biological Control Agent of Leafy Spurge

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### Abstract

Leafy spurge, *Euphorbia esula* L., a complex of species of Eurasian origin, is an aggressive deep-rooted perennial weed in rangelands of North America. A biocontrol project against this weed started in 1962. Since then 17 insect species were released in order to control leafy spurge in USA and Canada. In particular, six species of the genus *Aphthona* have been released and five of these are established (Julien, 1992; Gassmann and Schroeder, 1995; Gassmann, 1996; Gassmann *et al.*, 1996). However, economic losses caused by this noxious weed are still extremely high (see Gassmann *et al.*, 1996). For this reason an exploration for new biocontrol agents was conducted in 3 regions of Russia (Northern Caucasus, Western Siberia, Eastern Siberia) during the summer of 1998. Biological observations of *Aphthona russica* sp. nov., found in the Krasnodar territory of North West Caucasus, were made to evaluate its potential to control leafy spurge in North America.

### Material and methods

During late June, 1998, 70 female adults of *A. russica* were collected in the Taman Peninsula (Krasnodar territory, Northern Caucasus; 45° 19' N, 36° 51' E). The insects were kept in plastic cages and were provided with leafy spurge in bouquets for food. Preliminary biological observations on adults and larvae were carried out at the Biocontrol Group of the Zoological Institute, St. Petersburg, Russia, under laboratory conditions. Plants and beetles were illuminated by special fluorescent ballast lamps DRLF-400 suitable for photosynthesis (L:D = 12:12), with a temperature range from 20-22°C at night to 25-27°C during daytime. Two biotypes of *E. esula* were used to feed adults and larvae, from Krasnodar territory and from USA, respectively as control and test plants.

In total, more than 1000 eggs were laid under laboratory conditions and used to gather preliminary information on the biology of *A. russica*. Three main methods of rearing were tested:

- 1) Ovipositing females were placed in 10cm diameter Petri dishes, each containing a small bouquet of *E. esula* with several leaves. Females laid most of their eggs on leaves and on the bottom of the dish. Every second day, host plant leaves were replaced and laid eggs were transferred with a fine, soft, moistened brush to moist filter paper in a sterile, 4cm diameter Petri dish wherein eggs developed. When eclosion began, from

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10 to 25 neonate larvae were transferred with a brush to the stem base of *E. esula* potted plant (10cm diameter). Four replications were made. First instar larvae moved crawling down along the stem and roots into the soil, where their development occurred. Each month, the root system was inspected to record larval survival and development. To observe larval feeding behavior, some neonate larvae were transferred on *E. esula* roots placed on moist sterile sand in two 10cm diameter Petri dishes, and inspected daily.

- 2) Adults were kept and fed as in the previous method, but all laid eggs were transferred directly to the stem base of the plant. To maintain high humidity during egg development, each potted plant was covered with a plastic cylinder with ventilation holes. When larvae eclosed, they penetrated into the soil and were regularly observed as in the first method.
- 3) Adults (4-5 per plant) were kept on intact living potted plants covered with plastic cylinders closed by a gauze lid. Each 4-6 days, beetles were transferred to a new plant, while old plants with laid eggs were treated as in the second method.

## Results and discussion

In natural conditions *A. russica* typically occur in relatively humid habitats between forests and fields and in small valleys. Only females of this species were collected.

No significant feeding preference was observed when *A. russica* adults were fed on U.S. and Northern Caucasus 'biotypes' of *E. esula*. Oviposition occurred until August 11, 1998. Adults died soon after the period of oviposition. To estimate fecundity, four females were placed in Petri dishes and monitored for 35 days (July 2 - August 7). Mean fecundity and SEM over the period of observation was  $3.9 \pm 0.5$  eggs/female/day. This is similar to *A. cyparissiae* (Koch) females which laid an average of 285 eggs during three months (Gassmann *et al.*, 1996).

The mean time required for embryonic development at room temperature (ca 22°C) was  $18.8 \pm 0.6$  days. Observations of larvae in Petri dishes and in jars showed that newly hatched larvae crawled down along the stem and started feeding on root hairs. Two weeks later, the larvae began boring into the roots. In the laboratory, the larvae continued feeding for four months, showing very slow growth and development. The mature larvae left the roots, moved to the bottom of the jar, starting a prepupa-like period. It is clear that *A. russica* diapause at this stage. It is probably that such a slow egg and larval development is connected with the larval diapause. For example, in three *Aphthona* species with overwintering prepupae (*A. cyparissiae*, *A. flava* Guillebau and *A. czwalinae* Weise) studied by Gassmann *et al.* (1996), the mean duration of embryonic development was 17-19 days at 20°C, and the total larval feeding period lasted 2-4 months, which is quite similar to *A. russica*. However, flea beetle species that overwinter as adults usually exhibit much faster development. For example, in *A. abdominalis* Duftschmid, at approximately the same temperature the hatching period was 4-7 days and larval development required 18-20 days (Fornasari, 1993). Similar results were also obtained in our laboratory with *Aphthona pygmaea* Kutschera (unpublished data). In the root-feeding larvae of a closely related taxon, *Longitarsus albineus* (Foudras), the average time taken for the development of eggs and larvae was 12 and 32 days respectively at 20-22°C (Huber, 1979).

To compare different methods of rearing, the survival percentage from egg to prepupa-

pa stage was calculated. With the first method (see Materials and methods), 30% of newly hatched larvae transferred to the plant survived to the prepupa stage ( $n=26$ ). However, the eclosion percentage in Petri dishes was 13% ( $n=242$ ). Thus, total survival with this method was ca. 4%.

With the second method, the number of living prepupae represented 24% of the number of newly laid eggs transferred to the plant ( $n=62$ ).

With the third method, the survival percentage cannot be directly estimated, because the exact number of laid eggs is unknown. However, by multiplying the mean fecundity (3.9) with the number of females used (5 ex.) and with the total duration of the experiment (15 days), we conclude that approximately 300 eggs were laid. In total, 22 prepupae were found, representing ca. 7% survival.

Unfortunately, such a low percentage of survival is rather typical for laboratory rearing of root-feeding larvae. For example, very high larval mortality (up to 95%) in laboratory conditions was recorded in another flea beetle already used for biocontrol of leafy spurge, *Aphthona flava* (Pemberton and Rees, 1990). In *A. venustula*, the percentage of survival of newly hatched larvae transferred to the stem base of potted plants ranged from 10 to 40% depending on the *Euphorbia* species, with 11% for the leafy spurge (Gassmann, 1996).

To select the optimal conditions for diapause and reactivation, in December the prepupae were divided into three groups and transferred in 3 different temperature regimes (five replications were made for each): 1) 4 months at 5°C; 2) 4 months at 18°C; and 3) 3 months at 5°C, plus 1 month at 25°C. In all regimes, pupae were not found until the end of March, when all larvae were transferred to green house conditions (mean temperature, ca. 25°C). The first pupations occurred in April by larvae of the first variant (from December through March, stored at 5°C); the first adults eclosed on May 8, 1999, and started feeding intensively, and in five days the first eggs were laid.

## Conclusions

- 1) *A. russica* is a monovoltine species with a diapausing prepupal stage.
- 2) The second method of rearing (eggs transferred to the potted plant) could be recommended for further studies on *A. russica* host specificity; however, in large scale experiments, the third method (adults ovipositing directly on the plant) is much less laborious.
- 3) Available data suggest that *Aphthona* species fall into two distinct groups based on their life history. The species of the first group develop slowly and overwinter as prepupae. The species of the second group develop fast and overwinter as adults.

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