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The biological control of leafy spurge (*Euphorbia esula* L.) in North America: Work done in Europe 1994-1998

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(*Article begins on following page.)

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FINAL REPORT

The biological control of leafy spurge (*Euphorbia esula* L.) in North America

Work done in 1994-1998

André Gassmann

June 1999

CABI Bioscience *integrating*:

IIBC	International Institute of Biological Control	IIE	International Institute of Entomology	IIP	International Institute of Parasitology	IMI	International Mycological Institute

Executive summary

Following the successful release of several *Aphthona* species targeted for dry and mesic leafy spurge habitats, studies were focussed on species from mesic-moist and shaded sites. Investigations on *A. venustula* and *A. ovata* started in 1991. Although most of the work was terminated in 1994 and 1995 respectively, screening tests have not yet been fully completed because of an increasing concern over potential feeding on native "threatened and endangered" *Euphorbia* species, and the lack of native spurge species for host specificity studies. In 1995, work started on *A. violacea*, a continental species exclusively associated with humid habitats. This work has not yet been completed for the same reason.

Both *A. venustula* and *A. ovata* are the best suited European *Aphthona* species for shaded habitats. *Aphthona venustula* has a broad macroclimatic range within its European distribution range whilst *A. ovata* is best adapted to subconmtinental and mountain areas. The three species have in common that they overwinter as adults, and *A. violacea* has a partial second generation in the laboratory. For all three species, normal adult feeding and longevity in no-choice tests occurred on nearly all spurges in subgenus Esula. Adult feeding and prolongued longevity occurred on some spurge species in subgenera Agaloma, Chamaesyce, Euphorbium, and occasionally Poinsettia. Slight adult feeding and little prolongued longevity occurred on some species in family Euphorbiaceae as well, in particular with *A. violacea*. For all three species, the range of plant species accepted for adult feeding is considerably wider than that of species suitable for completion of larval development. All species in subgenus Esula are to some degree suitable host plants for larval development. Larvae can develop successfully on species in subgenus Agaloma (e.g. *E. discoidalis*, *E. marginata*, and in particular *E. corollata*), and rarely in subgenera Euphorbium (*E. milii*, *E. tirucalli*) and Poinsettia (*E. heterophylla*).

Given the results obtained so far with these three *Apththona* species, it is recommended to make additional tests with, 1) other species in subgenus Agaloma like *E. hexagona* (annual, sympatric with leafy spurge), *E. nephradania* (annual, sympatric with leafy spurge), *E. strictior* (perennial, possibly sympatric with leafy spurge), *E. zinniiflora* (perennial, sympatric with leafy spurge in eastern USA), *E. ipecacuanhae* (perennial, sympatric with leafy spurge in eastern USA), or *E. exserta* (perennial), 2) to test perennial species in subgenus Chamaesyce like *E. fendleri*, *E. polycarpa* (both sympatric with leafy spurge) or *E. deltoidea* which is under review for legal protection, and 3) to test endangered or threatened species in subgenus Esula, i.e. *E. purpurea* and *E. telephiodes*. *Euphorbia purpurea* is of particular importance since it is sympatric with leafy spurge in eastern USA and it occurs in moist habitats. In case these species may be to difficult to obtain, they could be replaced by more common ones in subgenus Esula like *E. inundata* or *E. robusta*.

In biocontrol of leafy spurge, the root-feeders will be supplemented with insects, such as species in genera *Oberea* and *Pegomya* which damage the shoot and prevent flowering, thus reducing the long distance dispersal of the weed. Studies on species in genus *Pegomya* have been completed in 1993 with the result that only *P. euphorbiae* is compatible with leafy spurge in North America. In 1995-96 work was carried out on *Oberea moravica* sp.n. collected on *E. lucida* in humid sites. Heavy to moderate adult feeding occurred on spurge species in subgenera Esula, Agaloma and Euphorbium. Although oviposition occurred only occasionally on species outside subgenus Esula, several species outside the weed subgenus may well support larval development. Within genus *Euphorbia*, it seems that plant species with thick and hollow stems such as *E. platyphyllos* (subgenus Esula) and *E. marginata* (subgenus Agaloma) are preferred hosts for larval development. Given these results and the low impact observed on the field host plant, *O. moravica* was discarded as potential biological control agent of leafy spurge in the humid habitats for which it was selected in North America.

Although all food niches and habitats may be filled with European insects, some species, such as *O. erythrocephala*, failed to establish or to build up high population densities possibly due to a lack of climatic matching, and some others, such as *C. hungarica* are expected to do so because they are not fully compatible with Canadian leafy spurge. This is the reason why *O. donceeli* and *Chamaesphecia* spp. from Northern China were studied with the expectation that east-Palearctic spurge insects will be better pre-adapted to leafy spurge and to the northern distribution of the weed. Studies conducted in 1992-93 showed that the larvae of *C. schroederi* sp.nov. did not develop in the roots of leafy spurge indicating that east-Palearctic *Chamaesphecia* species may also be very specific to their field hosts. From 1993 to 1995, another *Chamaesphecia* species – closed to *C. tenthrediniformis*- was tentatively collected in China and studied. No larval development occurred on North American leafy spurge from the 60 larvae which could be reared and transferred on the target weed.
Between 1994-96, host range studies was carried out with *O. donceeli* from Inner Mongolia on 27 plant species. Moderate to heavy adult feeding was observed on North American leafy spurge, and on most spurge species in subgenus Esula and Agaloma, i.e., *E. lathyris*, *E. platyphyllos*, *E. polychroma, E. incisa*, *E. corollata*, and *E. marginata*. Moderate feeding was recorded on *E. tirucalli*, *E. pulcherrima*, *E. heterophylla*, and outside family Euphorbiaceae, on *Asclepias syriaca.* Although oviposition occurs only occasionally on species outside subgenus Esula, several species outside this subgenus may well support larval development (e.g. *E. marginata*) and to a lesser extend *E. tirucalli*. As for *O. moravica*, it is unclear to which extend leafy spurge is a prefered host for *O. donceeli*.
In conclusion, none of the new species studied for biological control of leafy spurge in humid habitats (*O. moravica*), or harsher climats (*O. donceeli* and *Chamaesphecia* spp. from China) do show good prospect. Completion of the host range studies with *A. venustula*, *A. ovata* and *A. violacea* has been impeded because no additional native spurges became available in spite of numerous attemps to obtain seeds or plant material. Foreign exploration on potential biological control agents of leafy spurge has been interrupted in 1999. Completion of the screening tests of the above mentionned *Aphthona* species will be hopefully completed in 2000 when the native spurge will be available.

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1. Introduction

Leafy spurge (*Euphorbia esula* (s.l.) is a toxic herbaceous perennial of Eurasian origin that has become a serious weed on the North American prairies but it is also found along roadsides, river banks, flood plains and mountain slopes. Cultural and chemical control methods have proved expensive, largely ineffective on the long term and ecologically undesirable. Leafy spurge is not a serious weed in Eurasia, where it is attacked by a large complex of specialized insects and pathogens It is therefore a suitable target weed for classical biological. During surveys made in Europe nearly 40 specialized insect species and two pathogens species have been found on *E. esula* (s.l.) and related species and considered for the biological control of leafy spurge. Following the success of the *Aphthona* species released in Canada between 1982 and 1985, the strategy in the biocontrol of leafy spurge was centered on this group of agents, using rust fungi and species in genus *Chamaesphecia* to complement the impact of the leaf beetles. Because leafy spurge occurs in a wide range of habitats, the purpose of the action was also to fill the root-feeding guild of leafy spurge in the four main habitats of the weed, i.e. the dry open, mesic open, moist and shaded habitats.

With exception of the rust fungi the potential of which could not be realized, the strategy and work programme fixed in 1987 has been successfully commenced. In 1990, *A. lacertosa*, a species adapted to mesic-moist habitats was released in Canada. The study and screening tests with the root-boring species *C. hungarica*, *C. astatiformis* and *C. crassicornis* have been completed between 1990 and 1993. *Chamaesphecia hungarica* is suited for mesic-moist and shaded habitats, *C. astatiformis* for open mesic, and *C. crassicornis* for open mesic-dry habitats. The host range of all three species is restricted to subgenus Esula in genus *Euphorbia*. *Chamaesphecia hungarica* and *C. crassicornis* have been continuously released in North America since 1992 and 1994 respectively. After an attempt in 1993, the release of *C. astatiformis* has been interrupted because of the low acceptance rate of this species on North American leafy spurge.

Studies on *A. venustula* and *A. ovata* started in 1991. *A. venustula* is a species of mesic and mesic-dry forests and permanent pastures on land cleared from forest. *A. ovata* is best adapted to mountain areas on mesic permanent pastures and can spread into forests. Both species overwinter as adults, attack in early spring and complete development by July. This life cycle enables the two species to survive in the short growing season at the northern leafy spurge limit or in mountain areas. A petition to release *A. venustula* was submitted in 1995. Reviewers rejected permission for field release of this species, because of concern over potential feeding on native “threatened and endangered “ *Euphorbia* species. Publication in 1996 of the Final Report on investigations in Europe on the life history and host range of *A. ovata* was postponed because the host range of this species is similar to that of *A. venustula.* Between 1995 and 1998, additional tests have been repeated with both species, but no additional critical North American native *Euphorbia* species could yet been tested. In 1995, work started on *A. violacea*, a continental species exclusively associated with humid habitats.

In biocontrol of leafy spurge, the root-feeders will be supplemented with insects, such as species in genera *Oberea* and *Pegomya* which damage the shoot and prevent flowering, thus reducing the long distance dispersal of the weed. Studies on species in genus *Pegomya* have been completed in 1993 with the result that only *P. euphorbiae* is compatible with leafy spurge in North America. The host range of *P. euphorbiae* is restricted to species in subgenus *Esula*. In 1995-96, work was carried out with *Oberea moravica* sp.n. collected on *E. lucida* in humid sites.

Although all food niches and habitats may be filled with European insects, some species, such as *O. erythrocephala*, failed to establish or to build up high population densities possibly due to a lack of climatic matching, and some others, such as *C. hungarica* are expected to do so because they are not fully compatible with Canadian leafy spurge. This is the reason why *O. donceeli* and *Chamaesphecia* spp. from Northern China were studied with the expectation that east-Palearctic spurge insects will be better pre-adapted to leafy spurge and to the northern distribution of the weed. Studies conducted in 1992-93 showed that the larvae of *C. schroederi* sp.nov. did not develop in the roots of leafy spurge indicating that east-Palearctic *Chamaesphecia* species may also be very specific to their field hosts. From 1993 to 1995, another *Chamaesphecia* species – closed to *C. tenthrediniformis* - was tentatively collected in China and studied.

2. Technical Report

2.1 Aphthona venustula

2.1.1 Taxonomy

Order:	Coleoptera
Family:	Chrysomelidae
Subfamily:	Haliticinae
Tribe:	Aphthonini
Genus:	Aphthona Dejean 1835
Species:	*A. venustula* Kutschera

The *A. venustula* populations used in this study were identified by M. L. Cox (CABI Bioscience). Voucher specimens are deposited in the CABI Bioscience Centre at Delémont, Switzerland and the CNC Ottawa, Canada.

Keys to the Halticinae genera (Heikertinger, 1925; Arnett, 1963; Scherer, 1969; Mohr, 1966; Balsbaugh and Hays, 1972) require that the elytral punctuation be irregular. This is true for the Palearctic species, but at least two of the North American species and many species elsewhere have feebly, but distinctly striato-punctate elytra (Maw 1981) and probably should be ascribed to another genus. The only key to the North American species is that by Horn (1889).

From the 27 *Aphthona* spp. recorded in Central Europe (Mohr, 1966), eight are distinctly large species (2.5-4 mm in length) while the remaining are small (1.5-2.4 mm in length). *Aphthona venustula* is a black species and about 1.8-2.4 mm long. It can be distinguished by a bicoloured black and brown front and mid femur and well pronounced shoulders. The species can easily be identified using the key provided by Mohr (1966).

2.1.2 Geographical distribution

According to Heikertinger (1944), *A. venustula* occurs in Europe between latitudes 40 N and 54 N. It is replaced in Sicily and the southern Balkans by *A. venustula* ssp. *attica* Weise which can be distinguished by its very light coloured front and mid femur (Heikertinger, 1944). *A. venustula* is not known in the Iberian Peninsula, Kazakhastan and Central Asia (Lopatin 1984).

2.1.3 Host plants and beetle site requirements

Aphthona venustula is a common species in hilly areas and the most abundant *Aphthona* species in wooded and transitional areas in western Europe, where it commonly occurs on wood spurge, *Euphorbia amygdaloides*, in mesic to mesic-dry south-exposed forest margins, and open woods. It also occurs on *E. cyparissias* in drier transitional areas, such as the Upper Rhine Valley. *Aphthona venustula* was rarely found on *E. cyparissias* and *E. esula* (s.l.) in eastern Austria and Hungary (Maw, 1981), but it is common in eastern Serbia, where it was found along the margins of mesic woodland on *E. amygdaloides*, and in open meadows on mainly *E. salicifolia* and *E. cyparissias* (I. Tosevski, pers. com.). It is also commonly found on *E. characias* in Italy (G. Campobasso pers. com.)
Spurges recorded at seven sites near Delémont were *E. amygdaloides* (6 sites), *E. cyparissias* (6 sites), *E. dulcis* (3 sites), *E. verrucosa* (2 sites) (all perennial species), *E. stricta* (5 sites) and *E.*

platyphyllos (cultivated land, 1 site) (both annuals*). Aphthona venustula* was found most abundantly from mid-April until early May on *E. amygdaloides* and *E. cyparissias* growing along forest margins, but beetles were collected on cypress spurge growing in open pastures as well. A few beetles were collected in early spring on *E. dulcis* (1 site, partly shaded), *E. verrucosa* (1 site, open) and *E. stricta* (1 site, partly shaded), but feeding marks (a small number) were only found on *E. verrucosa*. No *A. venustula* or feeding marks were found on *E. platyphyllos* invading a rape field. The beetle avoids very wet and very dry sites and preferably occurs on loamy soils and in dense vegetation. In the Centre's garden, a few adults of *A. venustula* were found in the open on North American leafy spurge, *E. amygdaloides*, *E. cyparissias*, *E. lucida*, *E. myrsinites*, but not on *E. polychroma* and *E. lathyris*. Maw (1981) found a few beetles on *E. stricta* in one of the seven sites inspected in western Europe. *Aphthona venustula* was not recorded on *E. helioscopa* at 11 sites in Central Europe.

2.1.4 Materials and methods

2.1.4.1 Life history

Field observations were made in the Delémont area, but quantitative beetle density was not assessed since the sites and spurges could not be swept with equal efficiency. Laboratory investigations were carried out with a Swiss population collected near the Centre on groups of 10-20 beetles were kept in transparent plastic cylinders (height 16 cm/diameter 11 cm) closed by a gauze lid. Shoots of *E. amygdaloides* were inserted into moist blocks of florist's sponge as an oviposition substrate. The newly hatched larvae were transferred to the stem base of potted greenhouse plants. In mid June the plants were covered with gauze bags to capture emerging beetles. The soil of all control and potted plants was kept in an outdoor shelter in individual photocollectors to ensure that all beetles had emerged and had been captured in the gauze bags during summer. Overwintering beetles were kept in an outdoor shelter in transparent plastic cylinders half filled with soil.

2.1.4.2 Completion of reproductive diapause

The need for a cold period to break reproductive diapause was determined with 120 females and 96 males that emerged between 6 July-4 August 1994. These were kept in groups of ten females and eight males in transparent plastic cylinders (height 16 cm/diameter 11 cm with a gauze lid), and a shoot of *Euphorbia amygdaloides*, inserted into a moist block of florist's sponge. The treatments were 6 and 12 weeks at 15 °C (13h light; 11h dark), 10 °C (11h light; 13h dark) and 5 °C (9h light; 15h dark) with two replicates per treatment. Subsequently, the beetles were kept at a 20 °C/12 °C cycle (15h light; 9h dark). The boxes were checked every ten days, the beetle mortality and oviposition noted, and the plants replaced.

2.1.4.3 Host range

Adult starvation tests.
Groups of 20 beetles were kept in transparent plastic cylinders (height 16cm/diameter 11cm) closed by a gauze lid, and a test plant shoot inserted into a moist block of florist's sponge was offered. The tests were run in the laboratory. The boxes were checked twice a week, the feeding response registered, the beetle mortality noted and the test plants replaced. The tests included 56 plant species.

Multiple-choice adult feeding tests.

Multiple-choice tests were made with test plants on which extended feeding occurred in starvation tests. Each of the 27 test plants together with *E. amygdaloides* was exposed to 20 beetles for four days in five simultaneous replicates.

Larval starvation tests.
Newly hatched larvae were transferred in May to the stem base of potted greenhouse test plants which were covered in mid June with a gauze bag to retain the emerging beetles. A minimum of 150 L1 larvae were used in each of at least three replicates on each test plant species. Larval transfers were made on 56 plant species.

Open field test.
Potted plants were arranged within a circle of four meters in diameter in the following way: three *E. amygdaloides* (control plants) were buried in the center of the circle and three pots each of nine test plant species were arranged symmetrically in concentric circles around the three control plants. The exterior boundary of the experimental plot consisted of a circle of five *E. amygdaloides* and five North American leafy spurge alternating with each other. On April 26, 1992, 450 adults of *A. venustula* were released in the centre of the circle (between the three control plants) and another 500 adults added three days later. The number of beetles resting on each plant was recorded on May 6, 12 and 19. The amount of adult feeding damage was evaluated on May 19 when the experiment was terminated. The plants were covered with gauze bags, kept in the greenhouse and the number of emerging adults recorded.

Field cage test.
The six test plant species used in this test were arranged in a 1x1x1 meter field cage in the following way: test plant species were randomly placed alternating with control plants in a 6x6 block design. Thus, the test comprised 3 replicates of each of the six test plant species and 18 control plants. The design was built up to offer a higher density of control plants than in the open field test. On May 7, 1992, 400 *A. venustula* were released into the cage. The test was terminated on May 30, and adult feeding damage evaluated. The plants were covered with gauze bags, kept in the greenhouse and the number of emerging adults recorded.

Oogenesis
Four replicates with five females each were kept for three weeks in transparent plastic cylinders (height 16 cm/diameter 11 cm) with a gauze lid, and the test plant shoot inserted into a moist block of florist's sponge. The following spurges were tested: *E. marginata*, (subgenus Agaloma), *E. lathyris*, *E. peplus*, *E. amygdaloides* (control) (subgenus Esula), *E. milii* (subgenus Euphorbium), *E. nutans* (subgenus Chamaesyce) and *Lythrum salicaria*, as well as a no food control. The boxes were checked twice a week, the beetle mortality and oviposition noted, and the test plants changed. The females still alive after three weeks were killed and dissected.

2.1.5 Results

2.1.5.1 Life history
Aphthona venustula has a single generation per year with overwintering in the adult stage. Around Delémont adults are found from early March to early November and were most abundant from early April to early May and the summer population is a mixture of spent parent beetles and reproductively immature F1 generation beetles. The number of sweep-netted beetles dropped drastically from the end of May onwards. About half of the beetles collected in mid April and kept on *E. amygdaloides*, were still alive in early July, the last beetle dying in early

October. First instar larvae transferred to greenhouse plants in early May developed to adults by July and there was less than 10% mortality until mid October. They did little feeding and usually did not mate or oviposit. All beetles emerged in summer and left the soil for some feeding since no beetles were found in the photocollectors the following year. Immature beetles overwintered in plastic containers and transferred to the laboratory in mid-March, had a survival rate of 62% (N=138) in 1991-92 and 52% (N=126) in 1992-93. The sex ratio of newly emerged beetles was nearly 1:1 (N=50) and was not changed by overwintering mortality. Seven (35%) of 20 beetles which could be collected in the field on 15 November were still alive on 15 March when kept in the outdoor shelter without any food. Three groups of ten females produced on average 40-50 eggs each per female. Single or small groups of eggs were laid underground near a host stem. Egg development took about two weeks at 21±1 °C.

2.1.5.2 Completion of reproductive diapause

Scattered oviposition may occur in summer, but most beetles are in reproductive diapause from summer until early spring. Oviposition started between 36-44 days after the end of the six weeks treatment the beetles were brought out of 5 °C, 10 °C, and 15 °C, and 15-27 days after a 12 weeks treatment at 15 °C and 10 °C, and after about 55 days on average after a treatment of 12 weeks at 5 °C. Between 18% and 100% of the total number of eggs laid on 15 March 1995 were laid within four weeks after onset of oviposition. Altogether, oviposition rate was low in all treatments, ranging from 2 to 14 eggs/female, and two replicates had no eggs laid. More eggs were laid after the 15 °C treatment (Ntot=244) than after the 5 °C treatment (Ntot=105).

2.1.5.3 Mortality factors

Aphthona venustula from three Swiss sites located in the same general area, one from Serbia and one from Italy were checked for disease. Thirty beetles from each site were killed just after collection or just after the shipment arrived and prepared on microscopic slides. Another ten beetles each of the same five populations were sent to J. Novotny (Forest Research Institute, Banska Stiavnica, Slovakia). Samples of the Swiss populations of *A. venustula* were also inspected after having spent three weeks in laboratory rearing boxes. With the exception of one *A. venustula* from Switzerland, no *Nosema* species (Protozoa) were found in the 1992 collections. However, gregarines (gut Protozoa) were very common in both the Italian and Serbian populations. They were rare in freshly caught Swiss beetles but common in beetles kept in the laboratory for three weeks. Gregarines are generally considered to be harmless to their hosts. Finally a very few pathogenic nematodes in the family Allantonematidae were found in one Swiss and the Italian population of *A. venustula*. These nematodes may cause mortality and/or reduce female fecundity. Thus, the beetles tested were not completely free from pathogenic microorganisms, but their populations do not suffer from epidemic diseases. This is confirmed by the results of the starvation tests which indicate prolonged longevity of beetles on their field host plants. No larval parasitoid were detected but *Microctonus* spp. (Braconidae) have been reared from other *Aphthona* spp. (Sommer and Maw, 1982).

2.1.5.4 Host range

Adult starvation tests.

Normal beetle feeding and longevity occurred on the field hosts *E. amygdaloides*, *E. salicifolia* and *E. cyparissias* as well as on *E. peplus* and *E. incisa* (Table 1). Half of the beetles survived 70-100 days, and the last ones died within 150-180 days. Moderate feeding but reduced longevity occurred on *E. marginata*, *E. corollata*, *E. milii*, *E. platyphyllos*, *E. helioscopa*, *E. seguieriana*, *Lythrum salicaria*, *E. myrsinites* and probably *E. spatulata* (test interrupted). On these plants half of the beetles survived 40-50 days and the last beetles usually died within 60-

90 days. Moderate feeding and a further reduced longevity was observed on several other species e.g., *E. maculata*, *E. lathyris*, *E. dulcis*, and probably *E. nutans* (tests interrupted). On these plants half of the beetles survived 20-30 days and the last beetles died within 40-70 days. Slight feeding and prolonged longevity was observed on a number of plant species like, *Ricinus communis*, *Manihot esculenta*, *Prunus* sp., *Sonchus arvensis* and *Vinca rosea*. On these plants half the beetles survived 20-25 days and the last beetles usually died within 50 days. On *Acalypha hispida*, *Rosa* sp. and *Lactuca satica*, half of the beetles died after about 10 days but a few survived up to 60 days. On all other plant species and when no food was provided, the beetles died within 15-25 days.

Larval transfer tests.

Larval survival of *A. venustula* on the control plant (*E. amygdaloides*) and North American leafy spurge was inconsistent but in the three years 1991 to 1993 the mean survival rate was similar on these two species, i.e. between 9% and 11%. Larval development to adult was restricted to species in subgenus Esula, as well as to *E. corollata* and to a lesser extend to *E. discoidalis* (subgenus Agaloma), of which one adult developed from 350 larvae transferred. In subgenus Euphorbium, one adult developed on *E. milii* (subgenus Euphorbium) from 400 larvae transferred (Table 1).

Table 1. Synopsis of host specificity test results for *Aphthona venustula* (1990-94/1998)

	Feeding by adults	Larval development to adults							
	No-choice starvation tests	# Larvae/ replicate		# replicates		# Replicates attacked		Mean % survival	
Euphorbiaceae	1990-94	1990-94	98	1990-94	98	1990-94	98	1990-94	98
Genus *Euphorbia*									
Subg. **Chamaesyce**									
* *E. maculata*	+	30	10	6	8	0	0	0	0
* *E. nutans*	+	20		7		0		0	
* *E. prostrata*	+	20		7		0		0	
E. chamaesyce			10		15		0		0
Subg. **Agaloma**									
* *E. marginata*	++	50	10	8	15	0	0	0	0
* *E. corollata*	++	50	30	7	5	0	2	0	4.0
* *E. discoidalis*	(+)	50		7		1		0.3	
* *E. antisyphilitica*	(+)	50		5		0		0	
Subg. **Poinsettia**									
E. pulcherrima	-	50		5		0		0	
* *E. heterophylla*	-	50		6		0		0	
* *E. cyathophora*	(+)	50		5		0		0	
Subg. **Euphorbium**									
E. tirucalli	(+)	50	50	5	3	0	0	0	0
E. milii	+	50	30	5	5	1	0	0.4	0
Subg. **Esula**									
E. lathyris	+	50		3		3		26.7	
E. polychroma	(+)	50		6		1		1.0	
E. helioscopa	++	50		3		3		16.7	
E. platyphyllos	+	50		3		3		7.3	
E. peplus	++	50		3		3		36.0	
* *E. spatulata*	++								
* *E. incisa*	++	50		11		2		0.4	
E. cyparissias	++	50		4		4		30.0	
E. seguieriana	+	50		5		4		5.2	
E. amygdaloides	++	50	30	48	12	44	12	9.3	24.2
N-A- Leafy spurge	++	50	30	24	8	21	6	11.1	7.1
E. myrsinites	+	50		5		1		6.4	
Acalypha hispida	(+)	50		5		0		0	
Ricinus communis	(+)	50		6		0		0	
Mercurialis perennis	-	50		4		0		0	
Pedilanthus tithymal.	(+)	50		5		0		0	
Croton variegatum	-	50		5		0		0	
Manihot esculenta	(+)	50		5		0		0	
Linaceae									
Linum flavum	-	50		3		0		0	
Linum usitatis.	-	50		7		0		0	
Geraniaceae									
Geranium sp.	(+)	50		3		0		0	
Pelargonium sp.	-	50		3		0		0	
Cistaceae									
Helianthemum numul.	-	50		3		0		0	

	Feeding by adults No-choice starvation tests	Larval development to adults							
		# Larvae/ replicate		# replicates		# Replicates attacked		Mean % survival	
	1990-94	1990-94	98	1990-94	98	1990-94	98	1990-94	98
Rosaceae									
Rosa sp.	(+)	50	30	3	4	0	0	0	0
Prunus sp.	(+)	50	30	3	3	0	0	0	0
Iridaceae									
Iris sibirica	-	50		3		0		0	
Liliaceae									
Hemerocallis sp.	-	50		3		0		0	
Lythraceae									
Lythrum salicaria	+	50		6		0		0	
Apocynaceae									
Vinca rosea	(+)	50		3		0		0	
Vinca minor	-	50		3		0		0	
Asclepiadaceae									
Asclepias syrica	-	50		3		0		0	
Hoya bella	-	50		3		0		0	
Vincetoxicum hirundinaria	-								
Asteraceae									
Lactuca sativa	(+)	50	30	4	5	0	0	0	0
Sonchus arvensis	(+)	50		3		0		0	
Cichorium intybus	-	50		3		0		0	
Economic plants									
Chenopodiaceae									
Beta vulgaris	-	50		3		0		0	
Umbellifereae									
Daucus carota	-	50		3		0		0	
Convolvulaceae									
Ipomoea batatas	-	50		4		0		0	
Solanaceae									
Solanum tuberosum	-	50		3		0		0	
Cruciferae									
Brassica napus	-	50	30	3	5	0	0	0	0
Polygonaceae									
Rheum rhaponticum	(+)	50		3		0		0	
Graminae									
Zea mays	-	50		3		0		0	

Adult feeding damage:

* US native spurges

-- no feeding
(+) occasional or slight feeding
+ moderate feeding
++ heavy feeding

Multiple-choice adult feeding tests.

There was less feeding on most species outside subgenus Esula in genus *Euphorbia*, and only minute nibbling occurred on *Manihot esculenta*, *Lythrum salicaria*, *Prunus* sp., and *Sonchus arvensis* (Table 2). No feeding marks were recorded on *Ricinus communis*, *Acalypha hispida*, *Pedilanthus tithymaloides*, *Rosa* sp., *Lactuca sativa*, *Rheum rhaponticum* and *Vinca rosea.*

Table 2. Results of multiple-choice adult feeding tests with *Aphthona venustula*

Test plant species	**Amount of feeding in five replicates**				
Subgenus CHAMAESYCE					
E. sepyllifolia	(+)	-	(+)	-	-
Subgenus AGALOMA					
E. marginata	+	+	(+)	+	+
E. corollata (1991)	(+)	(+)	+	(+)	(+)
E. corollata (1992)	+	(+)	(+)	+	+
E. discoidalis	+	-	-		
E. antisyphilitica	-	(+)	(+)	(+)	(+)
Subgenus POINSETTIA					
E. cyathophora	-	-	-	-	(+)
Subgenus EUPHORBIUM					
E. milii	+	(+)	+	(+)	+
E. tirucalli	(+)	(+)	+	+	(+)
Subgenus ESULA					
E. lathyris	+	+	+	+	+
E. myrsinites	(+)	-	-	(+)	+
Leafy spurge	++	++	+	++	++
E. amygdaloides	++	++	++	++	++
E. spatulata	+	+	+	+	+
E. peplus	++	+	++	+	++
E. platyphyllos	+	+	+	+	(+)
E. helioscopa	+	+	+	+	+
Ricinus communis	-	-	-	-	-
Manihot esculenta	-	-	(+)	-	-
Acalypha hispida	-	-	-	-	-
Pedilanthus tithymaloides	-	-	-	-	-
Lythrum salicaria	(+)	-	(+)	-	-
Rosa sp.	-	-	-	-	-
Prunus sp.	-	(+)	(+)	-	-
Sonchus arvensis	-	-	-	-	(+)
Lactuca sativa	-	-	-	-	-
Vinca rosea	-	-	-	-	-
Rheum rhaponticum	-	-	-	-	-

Open field test.

A total of 627 beetles was recorded on May 6, 12 and 19, 1992 (Table 3) and beetles were found on all plant species exposed in the test. A single beetle was recorded on *E. nutans* (subgenus Chamaesyce) and 9 beetles on *E. milii* (subgenus Euphorbium). A total of 55 beetles were found on *E. marginata* and *E. corollata* (subgenus Agaloma). Within subgenus Esula, few beetles were found on *E. lathyris*, *E. myrsinites, E. incisa* as well as on leafy spurge. The largest number of beetles was recorded on *E. peplus*. Feeding marks were observed on all plant species. The amount of adult feeding damage was similar to that observed in no-choice and multiple-choice tests. A total of 95 adults emerged exclusively from species in subgenus Esula. The position of plants within the experimental circle had no influence on the parameters measured.

Table 3 : Open field test with *Aphthona venustula*

	Number of beetles recorded on plants					
Plant species	6 May	12 May	19 May	Total	Adult feeding	# adults emerged
E. nutans (CHAMAESYCE)	1	0	0	1	(+)	0
	0	0	0	0	-	0
	0	0	0	0	(+)	0
E. corollata (AGALOMA	3	3	0	6	+	0
	3	6	0	9	+	0
	3	1	0	4	+	0
E. marginata	15	8	3	26	+	0
	7	2	0	9	(+)	0
	0	1	0	1	(+)	0
E. milii (EUPHORBIUM)	0	1	0	1	(+)	0
	0	6	1	7	+	0
	0	1	0	1	(+)	0
E. peplus (ESULA)	8	51	71	130	++	5
	13	26	42	81	++	12
	4	23	8	35	++	3
E. platyphyllos	5	8	3	16	+	15
	8	11	14	33	+	0
	7	4	4	15	+	5
E. lathyris	0	6	2	8	+	6
	0	1	0	1	+	6
	6	1	9	8	+	3
E. myrsinites	1	2	0	3	(+)	0
	5	2	0	7	+	6
E. incisa	6	1	0	7	+	4
	2	0	2	4	+	3
	1	3	2	6	+	2
E. amygdaloides *	10	8	3	21	++	1
*	6	4	7	17	++	0
*	20	12	8	40	++	4
	8	14	4	26	++	9
	0	1	2	3	+	0
	0	0	0	0	+	0
	1	12	1	14	+	1
	4	20	19	43	++	5
Leafy spurge	8	1	0	9	+	4
	7	0	0	7	+	0
	1	0	1	2	+	0
	3	5	1	9	+	10
	6	3	0	9	+	6

* Control plants placed in the centre of the experimental plot

Field cage test.
Adult feeding was observed on all plant species offered (Table 4). In contrast to all previous tests, feeding was negligeable on *E. corollata* and *E. lathyris*. The 44 adults emerged exclusively from species in subgenus Esula.

Table 4: Field cage test with *Aphthona venustula*

Plant species	Adult feeding	# of adults emerged
E. marginata (AGALOMA)	(+)	0
	+	0
	-	0
E. corollata	(+)	0
	(+)	0
	+	0
E. lathyris (ESULA)	-	0
	(+)	0
	-	1
E. peplus	++	1
	+	1
	+	5
E. spatulata	(+)	0
	(+)	0
	+	1
E. incisa	+	0
	+	1
	+	0
E. amygdaloides	+	5
	+	2
	+	0
	++	0
	+	6
	+	4
	+	0
	+	2
	++	2
	+	3
	+	0
	(+)	0
	+	2
	(+)	2
	+	3
	+	0
	+	2
	+	1

Oogenesis
Residual oviposition occurred in all the tests, e.g. on *Lythrum salicaria* and in the no-food control (Table 5). This represents eggs that had matured at the time of field collection and does not indicate oogenesis on the test plant. Abnormal egg development was not observed for any of the replicates. Normal oogenesis, oviposition and female survival occurred on *E. amygdaloides* and on the annual spurge, *E. peplus*. Thus, *E. peplus* is confirmed as a laboratory host. About 50% of normal oogenesis and oviposition occurred on *E. lathyris* and *E. marginata* with reduced female longevity on the former species. Still less oogenesis was observed on *E. milii* and *E. nutans*.

Table 5 : Oogenesis tests with *Aphthona venustula*

Test plant	Mean # eggs/ Female/day (range)	# females alive after three weeks	Mean # eggs in dissected females
E. nutans (CHAMAESPHYCE)	0.16 (0.01-0.50)	13	0
E. marginata (AGALOMA)	0.95 (0.44-1.48)	18	1
E. milii (EUPHORBIUM)	0.37 (0.12-0.68)	17	0.4
E. lathyris (ESULA)	1.08 (0.49-1.60)	8	0.5
E. peplus	1.67 (1.43-2.02)	16	2.5
E. amygdaloides	1.88 (1.34-2.57)	18	2.5
Lythrum salicaria	0.06 (0.01-0.08)	14	0
No food	0.04 (0.02-0.05)	0	0

Total number of females used for each test plant = 20

2.1.6 Discussion
A. venustula is a species of mesic and mesic-dry forest and permanent pasture on land cleared from forest. It has one generation per year. All beetles emerge in summer to feed, but oviposition is rare. The reproductive diapause lasts for a minimum of three months. It can be broken by 3-4 months at 15 °C/13L followed by 20C/15L. Thus, the reproductive activity of the beetle is likely to start with warmer spring temperatures (and longer photoperiod ?) together with the beginning of a sustained feeding activity. No cold temperature is apparently necessary for completion of the reproductive diapause of the beetle, but the conditions for optimal oviposition after termination of the reproductive diapause are unknown.
The main adult feeding and oviposition activity, in April-May, occurs before growth of most plant species growing in the surrounding of wood spurge. Thus, *E. amygdaloides*, the stems of which flower early in their second year, dominate the beetle habitat and can easily be swept. The situation is different in July-August when the new generation appears, i.e. the newly growing shoots of wood spurge are overtopped by other plants. Unlike *E. amygdaloides*, *E. cyparissias* and *E. salicifolia* do not have winter persistent stems and leaves, but they still grow early in spring so that beetles can be collected in April on these two species as well. Leafy spurge in North America behaves in a similar fashion in woodland or former woodland, so it should be accepted as a host. In western Europe, neither *E. virgata* nor *E. esula* s.str. are common in hilly and woodland or former woodland areas, so they are rarely attacked.

The beetle has a broad macroclimatic range within its European distribution range being found from the Mediterranenan area, to northern and Central Europe. *A. venustula* is adapted to a wide variety of climates, ranging from Mediterranean to subatlantic, and subcontinental. It extends beyond the northern limit of *E. esula* s.str. and *E. virgata* in Europe. In North America the beetle will possibly extend slightly south to the 40 N latitude.

The range of plant species accepted for adult feeding is wider than that of species suitable for completion of larval development. Even in the presence of field host plants, adult beetles nibbled and fed on non-host plants. Feeding occurred on spurges, such as *E. marginata*, *E. corollata* (subgenus Agaloma), *E. milii* (subgenus Euphorbium), *E. lathyris* and *E. helioscopa* (subgenus Esula), that did not support normal adult life expectancy. There was usually less feeding on these species when exposed in the presence of a field host plant, but some feeding occurred even under experimental field conditions. The beetles on *E. incisa* were long lived, but only very few larvae survived on this species. In contrast, *E. lathyris*, *E. platyphyllos* and *E. helioscopa* supported larval development, but not normal adult longevity. This indicates that the factors supporting adult and larval survival are different. Substantial adult feeding was restricted to genus *Euphorbia*, but nibbling occurred on several species in other genera. Thus, it appears that chewing is necessary for host recognition while A. *nigriscutis* and the other spring emerging species investigated usually recognize hosts outside genus *Euphorbia* without chewing.
The results of larval development tests show that larvae can develop on most, if not all, spurge species in subgenus Esula. Normal oogenesis occurred on plants in subgenus Esula which support normal adult feeding and adult life expectancy (*E. amygdaloides* and *E. peplus*). This does not exclude the possible use as field hosts of species in subgenus Esula on which beetles showed reduced oogenesis, such as *E. lathyris*, since the larval survival rate is usually high on species in this subgenus.
Larval development occurred occasionnally on *E. corollata* (subgenus Agaloma), and one larva developped to adult each on *E. discoidalis* (Subgenus Agaloma) and *E. milii* (Subgenus Euphorbium). Spurges in subgenera suboptimal for adult feeding or adult longevity supported reduced oogenesis. Reduced oogenesis on species outside subgenus Esula combined with only occasionnal completion of larval development much reduces the probability of establishment of *A. venustula* on spurge species outside this subgenus. The oogenesis tests confirm that the host range of *A. venustula* is restricted to spurges within subgenus Esula. Since *A. venustula* is restricted to woodland and permanent pasture on cleared woodland, only early growing species in subgenus Esula that occur in these habitats are actually vulnerable to attack. The low occurrence of *A. venustula* on annual spurge species in Europe is rather due to habitat condition than to host plant preferences. Most annual spurges grow at sites with disturbed ground, i.e. outside the habitat of *A. venustula*. However, field observations at several sites in Switzerland indicate that the beetle, within its habitat, discriminates between spurge species in subgenus Esula.

Within its habitat in Europe, *A. venustula* discriminates between perennial spurges in subgenus Esula, as well as between perennial and annual spurges. Therefore, the field host range *of A. venustula* in Europe is narrower than its experimental host range. In North America, some adult feeding and larval development on non-favoured species in subgenus Esula and other subgenera growing in or near dense beetle populations is likely to occur.

2.2. *Aphthona ovata*

2.2.1 Taxonomy

Order: Coleoptera
Family: Chrysomelidae
Subfamily: Haliticinae
Tribe: Aphthonini
Genus: *Aphthona* Dejean 1835
Species: *A. ovata* Foudras 1860

The *A. ovata* populations used in this study were identified by the two authors. Voucher specimens are deposited in the CABI Bioscience Centre at Delémont, Switzerland.
From the 27 *Aphthona* spp. recorded in Central Europe (Mohr, 1966), eight are distinctly large species (2.5-4 mm in length) while the remaining are small (1.5-2.4 mm in length). *A. ovata* is a black species, about 1.6-2.2 mm long. It can be distinguished by the entirely bright front and mid femur, the absence of shoulders and little pronounced front humps. The species can be identified using the key provided by Mohr (1966).

2.2.2 Geographical distribution

According to Heikertinger (1944), *A. ovata* is a mountain species which occurs in the Alps and the Carpathian mountains. It extends to southern Poland, the northern Appennins and the Balkans east to Asia Minor. According to Gruev & Tomov (1984), the distribution of *A. ovata* extends up to the woodland steppes of western Russia. It is not known from central Asia and Kazakhastan (Lopatin, 1984).

2.2.3 Host plants and beetle site requirements

Aphthona ovata was not found in the Delémont area and in the Rhine Valley and was uncommon in all areas surveyed in eastern Austria, Hungary and Serbia. In eastern Austria it was found mainly on *E. cyparissias* growing at mesic sites within rather dense vegetation (Maw, 1981). *Aphthona ovata* is rare in the Hungarian prairie, and it was found only twice. In Serbia it was found in small numbers up to 800 m altitude on *E. polychroma* and *E. amygdaloides* growing in forests and forest margins, as well as on *E. cyparissias* and *E. salicifolia* in meadows. The beetle probably occurs at higher elevations. It was recorded once on an annual spurge, *E. stricta* (Maw, 1981). It is a subcontinental and mountain species from Central and south-eastern Europe. The beetle avoids very dry and very wet sites, and prefers mesic-submesic forests and meadows in formely forested land. *Aphthona ovata* was usually found together with *A. venustula*. It is probable that it should replace the latter species at higher elevation or in harsher conditions.

2.2.4 Materials and methods

2.2.4.1 Life history

Field observations were made in Serbia. Quantitative beetle density was not assessed since the sites and spurges could not be swept with equal efficiency. Laboratory investigations were carried out with a Serbian population collected in early spring. Groups of 20 beetles were kept in transparent plastic cylinders (height 16 cm/diameter 11 cm) closed with a gauze lid. Shoots of *E. salicifolia*, *E. cyparissias* or leafy spurge were inserted into moist blocks of florist's sponge serving as oviposition substrate. The newly hatched larvae were transferred to the stem base of potted plants. The infested plants were kept in the greenhouse and covered with gauze bags by mid-June to capture emerging beetles. The soil of all control and test plants was kept in an outdoor shelter in individual photocollectors to ensure that all beetles had emerged and had been captured in the gauze bags during summer. Overwintering beetles were kept in an outdoor shelter in transparent plastic cylinders half filled with soil.

2.2.4.2 Host range

Adult starvation tests.

Groups of 10-20 beetles were kept in transparent plastic cylinders (height 16cm/diameter 11cm) closed with a gauze lid, and a test plant shoot inserted into a moist block of florist's sponge was offered. The tests were run in the laboratory. The boxes were checked twice a week, the feeding response registered, the beetle mortality noted and the test plants changed. The tests included 51 plant species.

Multiple-choice adult feeding tests.

Multiple-choice tests were made with critical test plant species on which extended feeding occurred during starvation tests. Each of the 18 test plants was offered for four days together with *E. salicifolia* or *E. polychroma* to ten beetles. Five replicates were made simultaneously in the laboratory.

Larval starvation tests

Newly hatched larvae were transferred to the stem base of potted plants and the infested plants transferred to the greenhouse. All larvae were transferred in May, and the test plants covered with gauze bags by mid-June. Larval transfers were made on 52 plant species. A minimum of three replicates of 30 or 50 larvae each were used with each plant species. Larval transfers to control plants were made at regular intervals.

Field cage tests.

The test run in 1992 comprised three replicates of each of nine test plant species and six E. salicifolia used as control plants. The plants were arranged in three rows in a 200 x130 x 110 cm field cage. Each row comprised each one test plant species and two control plants. On April 24, 1992, 300 beetles were released into the cage. The test was stopped on May 20 and adult feeding damage evaluated. Eggs laid in the soil were not recorded. The plants, covered with gauze bags, were kept in the greenhouse and the number of adults emerging recorded. In 1998, two to four potted plants each of ten plant species and the control were randomly assigned into each of the five field cages (2 x 2 x 1m), and 120 beetles put into each cage. The tests were started on 23 April and were terminated on May 25. Adult feeding was assessed on 5 May, and the number of beetles sitting on the plants were recorded on 5,14 and 25 May. On May 25, all plants were cleaned from beetles and kept as in the previous experiment.

2.2.5 Results

2.2.5.1 Life history

In the laboratory, about half of the beetles collected mid April were still alive at the end of July and the last beetle died in late October. Adult beetles overwintered in plastic cylinders in an outdoor shelter were transfered to the laboratory in mid-March. Overwintering survival rate of adults reached 66% (N=164) in 1991-1992 and 68% (N=173) in 1992-1993. In the laboratory, the oviposition period lasted for three months. Females laid eggs singly or in small batches underground near the stem of their hosts. When fed on North American *E. esula*, average fecundity was 80-120 eggs per female based on three replicates of ten females each in 1992 and 1993. About two third of the eggs laid in spring hatched. Egg development took about two weeks at 21±1 °C. First instar larvae transferred on potted plants in early May and kept in the greenhouse reached the adult stage in July. The sex ratio of newly emerged beetles was nearly 1:1 (N=50). A total of 10% to 15% adult mortality was recorded from July until mid-October. Inconsistent oviposition occurred in summer in the laboratory by females of the new *A. ovata* generation, but no adults emerged from first instar larvae transferred in August (progeny of the new generation). Thus, there is probably only one generation per year with a certain overlap of parent and F1 beetles during summer. The sex ratio of newly emerged beetles was 1:1.

2.2.5.2 Mortality factors

In 1992, 50 *A. ovata* adults were killed just after the shipment arrived, prepared on microscopic slides and checked for disease. Another 10 beetles were sent to Dr. J. Novotny (Forest Research Institute, Banska Stiavnica, Slovakia). No *Nosema* species or nematodes were found in the 1992 collection. Gregarines (gut protozoa), most species of which are considered harmless to their hosts, were very common, probably due to crowded conditions. Gregarines were rare in freshly collected Swiss populations of *A. venustula*, but very common in beetles which had been kept in the laboratory for three weeks. Thus, gregarines seem to spread quickly under crowded conditions.

There was no evidence of pathogenic organisms and collected populations did not suffer from epidemic diseases. This is confirmed by the results of starvation tests which indicate prolonged longevity of beetles on their field host plants.

2.2.5.3 Host range

Adult starvation tests

Normal feeding and longevity occurred on North American *E. esula*, *E. salicifolia*, *E. cyparissias*, *E. seguieriana*, *E. amygdaloides* and *E. peplus* (Table 6). A. slightly reduced longevity was observed on *E. polychroma*, *E. incisa*, *E. myrsinites E. helioscopa*, *E. corollata* and probably *E. marginata* (test interrupted). Moderate feeding and further reduced longevity occurred on *E. tirucalli*, *E. milii* and *L. salicaria*. Much reduced longevity was observed on *E. maculata* and *B. napus* although the last beetles died within 100 and 70 days, respectively. Although extended feeding occurred on *E. lathyris*, longevity was drastically reduced. Slight feeding and very little prolongued longevity was observed on *R. communis*, *M. esculenta* and *R. rhaponticum*. Slight feeding without prolongued longevity occurred on a few plant species like *Geranium* sp., *Rosa* sp. and *Lactuca sativa*. All beetles died within 10-25 days in the no-food control.

Larval starvation tests

Occasional larval development may occur on *E. tirucalli* (subgenus Euphorbium), *E. heterophylla* (subgenus Poinsettia) as well as on *E. marginata* (subgenus Agaloma). *Euphorbia corollata* (subgenus Agaloma) can be considered as a suitable host plant for larval development. All species in subgenus Esula are potential host plants for larval development. (Table 6).

Table 6. Synopsis of host specificity test results for *Aphthona ovata* (1995 /**1997**/1998)

	Feeding by adults No-choice starvation tests		# Days to 50% adult mortality		# Days to 100% adult mortality		Larval development to adults: # Larvae/ replicate			# replicates			# Replicates attacked			Mean % survival		
Euphorbiaceae	1995	1998	1995	1998	1995	1998	1995	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98
Genus *Euphorbia*																		
Subg. **Chamaesyce**																		
* *E. maculata*	+		25		100		30	**30**		5	**8**		0	**0**		0	**0**	
* *E. nutans*	+		30		90		30			5			0			0		
* *E. prostrata*	+		30		90		20			4			0			0		
E. chamaesyce		++		75		150		**20**			**6**			**0**			**0**	
* *E. glytosperma*									20			6			0			0
Subg. **Agaloma**																		
* *E. marginata*	++		80		stopped		50/30	**20**	20	5/4	**8**	6	1	**0**	0	0.2	**0**	0
* *E. corollata*	++		75		140		50	**30**		7	**5**		2	**3**		1.1	**5.3**	
* *E. discoidalis*	(+)		10		20		50			5			0			0		
* *E. antisyphilitica*	(+)		15		25		75			2			0			0		
Subg. **Poinsettia**																		
E. pulcherrima	(+)		15		30		50			7			0			0		
* *E. heterophylla*	--		10		15		50	**30**	30	5	**5**	5	0	**2**	0	0	**1.3**	0
* *E. cyathophora*	+		15		25		50	**30**	30	3	**3**	3	0	**0**	0	0	**0**	0
Subg. **Euphorbium**																		
E. tirucalli	+		75		100		50		30	6		4	1		1	0.3		0.8
E. milii	++		70		100		50			7			0			0		
Subg. **Esula**																		
E. lathyris	++		35		45		50			3			3			38.7		
E. polychroma	++	++	80	35	140	85	50	**30**	30	6	**4**	**6**	5	**4**	6	29.7	**47.5**	64.4
E. helioscopa	++		70		150		50			3			3			28.0		
E. characias	++		70		110		50			3			3			38.0		
E. peplus	++		100		190		50			3			3			33.3		
* *E. spatulata*								**10**			**5**			**2**			**8.0**	
* *E. incisa*	++		50		150		50	**30**		4	**5**		3	**3**		4.0	**3.3**	
E. cyparissias	++		100		190		50			4			4			26.0		
E. seguieriana	++		100		160		50			3			1			4.0		
E. amygdaloides	++		80		200		50			6			5			7.7		

	Feeding by adults No-choice starvation tests		# Days to 50% adult mortality		# Days to 100% adult mortality		Larval development to adults											
							# Larvae/ replicate			# replicates			# Replicates attacked			Mean % survival		
Euphorbiaceae	1995	1998	1995	1998	1995	1998	1995	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98
N-A- Leafy spurge	++	++	100	80	190	150	50	**30**		12	**9**		11	**7**		26.5	**25.6**	
E. salicifolia	++		100		180		50			7			7			13.7		
E. myrsinites	++		80		130		50			3			2			16.0		
Acalypha hispida	--		10		15		50			3			0			0		
Ricinus communis	(+)		25		50		50			3			0			0		
Mercurialis perennis	--		10		15		50			3			0			0		
Pedilanthus tithymal.	(+)		10		15		50			3			0			0		
Croton variegatum	--		10		15		50			3			0			0		
Manihot esculenta	(+)		25		30		50			4			0			0		
Plants attacked by other species in genus *Aphthona*																		
Linaceae																		
Linum flavum	--		10		15		30			3			0			0		
Linum usitatis.	--		10		15		50			3			0			0		
Geraniaceae																		
Geranium sp.	(+)		10		20		30			3			0			0		
Pelargonium sp.	--		10		15		30			3			0			0		
Cistaceae																		
Helianthemum numul.	--		10		15		30			3			0			0		
Rosaceae																		
Rosa sp.	(+)		15		20		50			3			0			0		
Prunus sp.	--		10		15		30			3			0			0		
Iridaceae																		
Iris sibirica	--		10		15		30			3			0			0		
Liliaceae																		
Hemerocallis sp.	--		10		15		30			3			0			0		
Lythraceae																		
Lythrum salicaria	+		40		140		50			3			0			0		
Plants with latex in other families																		
Apocynaceae																		
Vinca rosea	(+)		20		30		30			3			0			0		

	Feeding by adults No-choice starvation tests		# Days to 50% adult mortality		# Days to 100% adult mortality		Larval development to adults			
							# Larvae/ replicate	# replicates	# Replicates attacked	Mean % survival
	1995	1998	1995	1998	1995	1998	1995 **97** 98	1995 **97** 98	1995 **97** 98	1995 **97** 98
Vinca minor	--		10		15		50	3	0	0
Asclepiadaceae										
Asclepias syrica	(+)		10		20		30	3	0	0
Hoya bella	--		10		15		30	3	0	0
Asteraceae										
Lactuca sativa	(+)		10		30		50	3	0	0
Cichorium intybus		(+)		10		15				
Economic plants										
Chenopodiaceae										
Beta vulgaris	--		10		15		30	3	0	0
Umbellifereae										
Daucus carota	--		10		15		30	3	0	0
Convolvulaceae										
Ipomoea batatas	--		10		15		30	3	0	0
Solanaceae										
Solanum tuberosum	--		10		20		30	3	0	0
Cruciferae										
Brassica napus	(+)	--	10	10	70	15	30	3	0	0
Polygonaceae										
Rheum rhaponticum	(+)		15		35		30	3	0	0
Graminae										
Zea mays	--		10		15		30	3	0	0
No food control			**10**	10	**20**	15				

Adult feeding damage:

-- no feeding
(+) occasional or slight feeding
+ moderate feeding
++ heavy feeding

* US native spurges

Multiple-choice adult feeding tests

There was slightly less feeding on all species in genus *Euphorbia* (except *E. incisa*; Table 7). Some nibbling was found on *M. esculenta* and only one nibbling was recorded on *P. tithymaloides*, *L. salicaria* and *Rosa* sp. No feeding was recorded on *E. cyathophora* (subgenus Poinsettia), *R. communis*, *L. sativa*, *B. napus*, and *R. rhaponticum*.

Table 7. Results of multiple-choice adult feeding tests with *Aphthona ovata*

Test plant species	**Amount of feeding in five replicates**				
Subgenus CHAMAESYCE					
E. maculata	-	-	-	-	-
E. nutans	(+)	(+)	(+)	+	(+)
Subgenus AGALOM					
E. marginata	+	+	+	+	+
E. corollata	(+)	+	+	(+)	+
Subgenus POINSETTIA					
E. pulcherrima	-	-	-	-	(+)
E. cyathophora	-	-	-	-	-
Subgenus EUPHORBIUM					
E. milii	+	(+)	(+)	(+)	(+)
E. tirucalli	+	(+)	(+)	+	+
Subgenus ESULA					
E. lathyris	+	-	-	-	+
E. incisa	++	++	++	++	++
Leafy spurge	++	++	+	++	++
Ricinus communis	-	-	-	-	-
Manihot esculenta	(+)	(+)	(+)	-	-
Pedilanthus tithymaloides	-	-	-	-	(+)
Lythrum salicaria		(+)	-	-	-
Rosa sp.	-	(+)	-	-	-
Lactuca sativa	-	-	-	-	-
Brassica napus	-	-	-	-	-
Rheum rhaponticum	-	-	-	-	-

-	no feeding
(+)	occasional or slight feeding
+	moderate feeding
++	normal feeding

Field cage tests

The rate of adult feeding observed on all test species was quite similar to that observed in no-choice and multiple-choice laboratory tests (Table 8,9). A total of 315 beetles emerged from the test plants. With the exception of a few beetles emerging from *E. corollata* and *E. marginata*, and one beetle from *E. tirucalli*, development to adult was restricted to species in subgenus Esula. The largest number of adults emerged from *E. salicifolia*, leafy spurge, and the annual species *E. peplus* and *E. lathyris*. Only a few beetles emerged from E. incisa.

Table 8. Field cage test with *Aphthona ovata* (1992)

	# of plants offered	Adult feeding	# adults emerged from plants (7/92)	# of plants attacked
Subg. CHAMAESYCE				
Euphorbia nutans	3	(+)	0	0
Subg. AGALOMA				
E. corollata	3	+	0	0
E.marginata	3	+	2	1
Subg. EUPHORBIUM				
E. milii	3	(+)	0	0
Subg. ESULA				
E. incisa	3	++	2	1
E. peplus	3	++	46	3
E. lathyris	3	(+)	18	3
E. myrsinites	3	+	3	1
E. amygdaloides	3	+	5	1
E. salicifolia	6	++	122	6

Table 9. Field cage test with *Aphthona ovata* (1998)

	Total	**Total # of adults recorded on plants and adult feeding**				**Larval development to adults**	
set up: 23 April 98 terminated: 25 May	# of plants offerred	# adults on May 5	# adults on May 14	# adults on May 25	Adult feeding on May 5	# adults emerged from plants (7/98)	# of plants attacked
Cages 1 & 2							
Euphorbia nutans	8	5	9	2	+	0	0
E. chamaesyce	8	4	0	0	(+)	0	0
E. glytosperma	8	0	0	0	--	0	0
E. serpylifolia	8	2	0	0	(+)	0	0
N-A leafy spurge	8	15	60	39	++	45	6
Cages 3 & 4							
E. corollata	10	6	11	10	++	16	4
E.marginata	12	16	18	7	++	0	0
E. cyathophora	8	4	2	1	--	0	0
E. tirucalli	4	2	6	7	--	1	1
E. milii	4	6	10	10	+	0	0
N-A leafy spurge	8	9	31	23	++	30	7
Cage 5							
E. incisa	4	15	21	9	++	3	2
E. spatulata	12	1	4	3	+	0	a)
N-A leafy spurge	4	14	7	6	+	46	4

a) 10 plants dying prematuraly were dissected, and three larvae were found in roots.

2.2.6 Discussion

A. ovata is a species of mesic and submesic forests and transitional areas. It is a species best adapted to subcontinental and mountain areas in Central and south-eastern Europe. Thus, it has a relatively narrow macroclimatic range within its European distribution range. *A. ovata* is a complementary species to *A. venustula* which it should replace in harsher conditions and at higher elevation. *Aphthona ovata* has one generation per year. All beetles emerge in summer to feed, but oviposition is unlikely to occur. The main adult feeding and oviposition activity occurs in April-May of the year following emergence. *Aphthona* ovata can be found on spurges which do not have winter persistent stems and leaves. Like those, leafy spurge in North America grows early in the season, so it should be accepted as a host. The life cycle of the beetle, and its adaptation to mountain areas should enable it to survive in North America in areas with a short growing season.

The range of plant species accepted for adult feeding is wider than that of species suitable for completion of larval development. Even in the presence of field host plants, adult beetles nibbled and fed on non-host plants. Generally, there was slightly less feeding on all species in genus *Euphorbia* (except *E. incisa*) in multiple-choice adult feeding tests. Some nibbling was found on *M. esculenta* and only one nibbling was recorded on *L. salicaria* and *Rosa* sp. No feeding was recorded on *E. cyathophora*, *R. communis*, *L. sativa*, *B. napus* and *R. rhaponticum*.

Although moderate adult feeding may occur on any species in subgenus Chamaesyce (e.g. **E. nutans*, **E. glytosperma*, **E. prostrata*, **E. maculata*, **E. serpylifolia* and *E. chamaesyce*), none of these species is able to sustain a new beetle generation. Occasional larval development may occur on *E. tirucalli* (subgenus Euphorbium). In subgenus Euphorbium, adult beetles prefer to feed on *E. milii* than on *E. tirucalli*, but no larval development to adult was recorded on the former species. Within subgenus Agaloma, normal adult feeding and longevity was recorded on **E. corollata* and **E. marginata*. **Euphorbia corrolata* can be considered as a suitable host plant for larval development, and occasional larval development may occur also on **E. marginata.* No larval development and less adult feeding were recorded on *E. discoidalis* and *E. antisyphilitica* in the same subgenus. All species in subgenus Esula are, to some degree, potential hosts of the flea beetle. **Euphorbia incisa* is a much less preferred host than leafy spurge, but it is probable that **E. spatulata* is a better host provided the plants are strong enough to permit larval feeding. Finally, it was noted that one larva developped to adult on *E. heterophylla* (subgenus Poinsettia), although species in this taxon usually suffer little from adult feeding. Outside genus *Euphorbia*, slight adult feeding was observed on *R. communis*, *M. esculenta*, as well as on *Rheum rhaponticum*, *Brassica napus*, *Geranium* sp., *Rosa* sp., and *Lactuca sativa* in other plant families. Moderate adult feeding and prolonged longevity occurred on *Lythrum salicaria*.

2.3 *A. violacea*

2.3.1 Taxonomy

Order:	Coleoptera
Family:	Chrysomelidae
Subfamily:	Haliticinae
Tribe:	Aphthonini
Genus:	Aphthona Dejean 1835
Species:	*A. violacea* Koch

From the 27 *Aphthona* spp. recorded in Central Europe (Mohr, 1966), eight are distinctly large species (2.5-4 mm in length) while the remaining are small (1.5-2.4 mm in length). *A. violacea* is a black species and about 2-2.4 mm long. It can be distinguished from *A. venustula* by a complete black front and mid femur and the shape of the aedeagus. The species can be identified using the key provided by Mohr (1966).

2.3.2 Geographic distribution

According to Heikertinger (1944) *A. violacea* occurs in Central Europe, southern France and northern Italy, the northern Balkans east to southern Russia and the Caucasus. The beetle has been recorded in Switzerland as well (Döberl, 1995). It is not known in Bulgaria (Warchalowski 1974), and in Kazakhastan and Central Asia (Lopatin, 1984).

2.3.3 Host plants and beetle site requirements

According to literature and our observations, *A. violacea* is exclusively associated with wet sites. It has been recorded mainly from *E. palustris* and *E. lucida*, two large spurge species growing in wet habitats. Maw (1981) found the beetle in Hungary on *E. virgata* (1x) and *E. platyphyllos* (2x) in wet sites as well.

2.3.4 Life history

In contrast to the univoltine species *A. venustula* and *A. ovata*, *A. violacea* has a partial second generation with hibernation in the adult stage. In the laboratory, about half of the beetles collected in April in the field and kept on the field hosts were still alive in early July, the last beetle dying in late summer. Larvae hatched from two third of the eggs laid in spring, and 15% of the first instar larvae transferred to greenhouse field host plants in early May developed to adults by late June-early July. The sex ratio of newly emerged beetles was nearly 1:1. Average fecundity of the first (summer) generation was 110 eggs (range 49-243; n = 10 individual pairs). Larvae hatched from two third of the eggs laid during summer, but less than 2% of the first instar larvae transferred to greenhouse field host plants from mid-July to late August developed to adults in September. The sex ratio of the second (autumn) generation was nearly 1:1 as well.

Eight out of the ten females and nine males of the first (summer) generation used in fecundity studies were alive by mid-November when they were transferred to hibernation conditions. One female only died during winter. All dead were replaced by beetles of the same age. Another 29 eggs on average (range 5-70; n = 10 individual pairs) were laid from March to July the following year, and all beetles died before autumn. To summarize, an average of 139 eggs (range 82-257) were laid by females of the first (summer) generation, 79% of which in summer of the same year, and 21% the following spring.

Ten out of 12 females of the second (autumn) generation were alive by mid-November when they were transferred to hibernation conditions, but did not lay any eggs. There was no overwintering mortality but two females died in early spring. Average fecundity was 102 eggs

(range 32-269; n = 8). Oviposition occurred between April and August, and all beetles died before autumn. In total, 65% of the beetles emerged in summer (first generation) survived until November, and 80% of those overwintered successfully. 90% of the beetles of the second (autumn) generation overwintered successfully.
In the field, *A. violacea* first appears on the plants in early March. By mid-April, the beetles strongly aggregate on the vegetative buds of the newly growing shoots. This period is accompanied by intensive feeding. Dispersal of the beetles starts when the host plant expands its leaves, so that by May adults can be found in small groups or individually feeding on leaves, or resting on the lower part of the stems. Intensive oviposition probably occurs in May. Egg batches are found at the stem base and in the soil. Only few beetles can be seen on the plants during summer.

2.3.5 Mortality factors
In 1995, a few Braconid adults (*Microctonus* sp. ?) were reared from the Hungarian and Serbian populations of *A. violacea*. In 1996, only a few parasitoids were reared from the Serbian population, but 15% of the Hungarian population was parasitized. No nematodes and only few gregarines were found in both populations.

2.3.6 Host range

Adults of *A. violacea* collected from *E. palustris* in Hungary and from *E. lucida i*n Serbia were used in the screening tests.

Adult starvation tests
Laboratory starvation tests were made with groups of 10-20 spring field collected beetles kept in transparent plastic cylinders closed by a gauze lid, and a test plant shoot inserted into a moist block of florist's sponge offered. The boxes were checked twice a week, the feeding response registered, the beetle mortality noted and the test plants replaced. The tests included 56 plant species and a no-food control.
Normal beetle feeding and longevity occurred on the field hosts *E. palustris* and *E. lucida*, as well as on North American leafy spurge (*E. esula* s.l.), *E. cyparissias* and *E. peplus* (Table 10). Slightly reduced adult feeding or longevity occurred on most other species in subgenus Esula like *E. incisa*, *E. spatulata*, *E. platyphyllos*, *E. amygdaloides*, *E. myrsinites* as well as on *E. nutans*, *E. maculata*, (subgenus Chamaesyce), *E. marginata*, *E. corollata* (subgenus Agaloma), and *E. milii* (subgenus Euphorbium). A further reduced longevity was observed on *E. lathyris*, and *E. prostrata*. Slight feeding and a much reduced longevity occurred on *E. pulcherrima*, *E. tirucalli*, *Manihot esculenta*, and *R. communis*, but one beetle survived 100 days on the latter species. Half of the beetles survived 25 days in the no-food control and the last individual dying only after 40 days.
Outside the family Euphorbiaceae, an unexpected adult behaviour has been observed. Moderate feeding and prolongued longevity of a few beetles occurred on *Rosa* sp., *Lactuca sativa*, *Daucus carota* and *Brassica napus*, and to a lesser extend on *Prunus* sp., *Cichorium intybus* and *Ipomea batatas*. Some variation on adult longevity occurs regularly between years on either plant tested. In 1998, the number of days to 50% adult mortality was reduced in most tests run as compared to 1997.

Larval starvation tests
Larval starvation tests were made with newly hatched larvae transferred in May to the stem base of potted plants which were covered with a gauze bag in mid June to retain emerging beetles. Emergence traps were unsuccessfully used for larval development tests in 1996. Usually a total

of 150 L1 larvae, or more, were used in several replicates on each of the plant species tested. The first adults emerged by the end of June and lasted for about three weeks. Larval development to adult was usually inconsistent on control plants and North American leafy spurge. Larvae can develop on most, if not all, spurge species in subgenus Esula, and the percentage of larval development to adults is hardly predictable. Larvae can develop successfully on species in subgenus Agaloma (*E. marginata*, and in particular *E. corollata*) but rarely in subgenus Euphorbium (*E. milii*). No larval development to adult was observed on species in subgenus Chamaesyce, and outside genus *Euphorbia*.

Table 10. Synopsis of host specificity test results for *Aphthona violacea* (1995 / *1996* / 1997 / 1998)

	Feeding by adults No-choice starvation tests				# Days to 50% adult mortality				# Days to 100% adult mortality				Larval development to adults: # Larvae replicate			# replicates			# Replicates attacked			Mean % survival		
Euphorbiaceae	1995	*96*	**97**	98	1995	*96*	**97**	98	1995	*96*	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98
Genus *Euphorbia*																								
Subg. **Chamaesyce**																								
* *E. maculata*	+	+			65	*50*			90	*100*			20			10			0			0		
* *E. nutans*	+				65				95				20			14			0			0		
* *E. prostrata*	+				50				70				20			5			0			0		
E. chamaesyce	+	+		++	35	*40*		55	--	*110*		100	20			10			0			0		
Subg. **Agaloma**																								
* *E. marginata*	+			+	55			35	100			65	20			10			3			2.0		
* *E. corollata*	++	++	**+**		50	*35*	**45**		80	*120*	**75**		30	**30**		4	**5**		2	**2**		5.8	**4.0**	
Subg. **Poinsettia**																								
E. pulcherrima	(+)		**(+)**		35		**20**		55		**35**		30			5			0			0		
* *E. heterophylla*	--		**--**		20		**15**		35		**40**				30			5			0			0
* *E. cyathophora*		(+)		(+)		*25*		20		*55*		35		**30**			**5**			**0**			**0**	
Subg. **Euphorbium**																								
E. tirucalli	+	+			40	*30*			60	*75*			30			5			0			0		
E. milii	+		**+**		40		**60**		100		**95**		30	**30**		5	**5**		0	**1**		0	**1.3**	
Subg. **Esula**																								
E. lathyris	++				50				60				30			7			6			13.8		
E. polychroma	+				50				100															
E. platyphyllos	++				65				100				30			5			3			4.0		
E. peplus	++				80				120				30			5			5			40.7		
E. helioscopa		++				*65*				*90*														
* *E. spatulata*	++				55				100				20	**10**	5	7	**15**	8	3	**3**	5	3.6	**3.3**	2.5
* *E. incisa*	++				65				100				30	**30**		4	**5**		1	**2**		1.7	**1.3**	
E. cyparissias	++				75				100															
E. amygdaloides	+				75				100															
N-A- Leafy spurge	++		**++**	++	75		**110**	55	120		**140**	120	30	**30**		17	**7**		15	**4**		13.7	**7.1**	
E. lucida	++	++	**++**		75	*75*	**105**		120	*120*	**135**		30	**30**		12	**9**		10	**8**		20.8	**7.4**	
E. palustris	++	++	**++**	++	60	*50*	**75**	50	130	*90*	**110**	140	30	**30**	30	12	**4**	7	12	**4**	7	10.0	**13.3**	32.4
E. myrsinites	+				80				90															

	Feeding by adults No-choice starvation tests				# Days to 50% adult mortality				# Days to 100% adult mortality				**Larval development to adults** # Larvae replicate			# replicates			# Replicates attacked			Mean % survival		
Euphorbiaceae	1995	*96*	**97**	98	1995	*96*	**97**	98	1995	*96*	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98
Ricinus communis	+	+	+		30	*30*	**30**		100	*60*	**65**		30			5			0			0		
Mercurialis perennis		(+)				*20*				*45*				**30**			**5**			**0**			**0**	
Pedilanthus tithymal.		(+)				*20*				*50*				**30**			**5**			**0**			**0**	
Croton variegatum		(+)				*20*				*45*				**30**			**5**			**0**			**0**	
Manihot esculenta		+	+			*40*	**40**			*50*	**45**		50			4			0			0		
Plants attacked by other species in genus *Aphthona*																								
Linaceae																								
Linum flavum		(+)				*20*				*35*				**30**			**5**			**0**			**0**	
Linum usitatis.	(+)	(+)	(+)		30	*35*	**25**		45	*55*	**55**		30			4			0			0		
Geraniaceae																								
Geranium sp.		+	+			*25*	**40**			*45*	**90**													
Pelargonium sp.		--				*20*				*40*														
Cistaceae																								
Helianthemum numular.		(+)				*20*				*35*														
Rosaceae																								
Prunus sp.		+	+			*35*	**35**			*50*	**45**			**30**			**5**			**5**			**0**	
Crassulaceae																								
Sempervivum sp.		--				*20*				*40*														
Iridaceae																								
Iris sibirica		(+)	(+)			*20*	**15**			*45*	**30**													
Liliaceae																								
Hemerocallis sp.		(+)	(+)			*20*	**20**			*35*	**30**													
Lythraceae																								
Lythrum salicaria		+	+			*45*	**75**			*110*	**105**			**30**			**5**			**5**			**0**	
Plants with latex in other families																								
Apocynaceae																								
Vinca rosea		--				*20*				*45*														
Vinca minor		--				*15*				*40*				**30**			**5**			**5**			**0**	
Asclepiadaceae																								
Asclepias syrica		(+)				*25*				*50*														
Hoya bella		--				*15*				*40*														

	Feeding by adults No-choice starvation tests				# Days to 50% adult mortality				# Days to 100% adult mortality				Larval development to adults: # Larvae replicate			Larval development to adults: # replicates			Larval development to adults: # Replicates attacked			Larval development to adults: Mean % survival		
	1995	*96*	**97**	98	1995	*96*	**97**	98	1995	*96*	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98	1995	**97**	98
Vincetoxicum hirund.		(+)				*15*				*30*														
Asteraceae																								
Lactuca sativa		+	+	++		*60*	**80**	40		*80*	**90**	80		**30**			**5**			**5**			**0**	
Cichorium intybus		+	+			*25*	**65**			*55*	**90**			**30**			**5**			**5**			**0**	
Economic plants																								
Chenopodiaceae																								
Beta vulgaris		--		--		*15*	**15**			*55*	**35**													
Umbelliferae																								
Daucus carota		+	+	+		*40*	**40**	30		*90*	**85**	45		**30**			**5**			**5**			**0**	
Convolvulaceae																								
Ipomoea batatas		+	+	+		*20*	**50**	20		*60*	**90**	80		**30**			**5**			**5**			**0**	
Solanaceae																								
Solanum tuberosum		--	(+)			*15*	**20**			*70*	**35**				30			3			0			0
Cruciferae																								
Brassica napus		+	+	+		*60*	**60**	35		*90*	**75**	80		**30**			**5**			**0**			**0**	
Polygonaceae																								
Rheum rhaponticum		(+)	(+)			*20*	**25**			*55*	**30**													
Graminae																								
Zea mays		(+)	(+)			*15*	**15**			*40*	**20**			**50**			**5**			**5**			**0**	
Asteraceae																								
Parthenium argent.		(+)				*20*				*30*														
No food control					30	*15*	**15**	15	40	*40*	**40**	20												

* US native spurges

Adult feeding damage:

- -- no feeding
- (+) occasional or slight feeding
- \+ moderate feeding
- ++ heavy feeding

Multiple-choice adult feeding tests.

Multiple-choice tests were made with test plant species on which extended feeding occurred in starvation tests. Each of the 28 test plant species was exposed simultaneously with *E. palustris* or North american leafy spurge to ten beetles for six days in four replicates. Adult feeding was checked twice.

There was slightly less feeding on all species in genus *Euphorbia* and minute nibbling was recorded two times on *R. communis* and *M. esculenta* (Table 11). There was much less feeding marks on all 15 plant species outside family Euphorbiaceae, particularly on *Rosa* sp., *Prunus* sp., and *Daucus carota*, but slight feeding occurred on *Lactuca sativa* in all replicates. No feeding marks were recorded on *Ipomoea batatas*, *Brassica napus* and *Zea mays*.

Table 11: Results of multiple-choice adult feeding tests with *A. violacea* (1995-96)

Test plant species	**Amount of feeding in five replicates**			
Subgenus CHAMAESYCE				
E. maculata	-	-	-	(+)
E. nutans	(+)	(+)	+	+
E. prostrata	-	(+)	(+)	-
E. chamaesyce	-	(+)	-	(+)
Subgenus AGALOMA				
E. marginata	+	(+)	-	-
E. corollata	+	+	+	+
Subgenus POINSETTIA				
E. cyathophora	-	(+)	-	-
Subgenus EUPHORBIUM				
E. milii	+	-	+	+
E. tirucalli	+	(+)	(+)	(+)
Subgenus ESULA				
E. lathyris	-	(+)	(+)	+
E. palustris	++	++	++	++
Leafy spurge	++	++	+	++
E. spatulata	++	++	++	+
Ricinus communis	(+)	(+)	-	-
Manihot esculenta	(+)	(+)	-	-
Linum usitatissimum	-	-	-	-
L. flavum	-	-	-	-
Geranium sp.	-	-	-	-
Rosa sp.	-	-	(+)	(+)
Prunus sp.	(+)	-	(+)	-
Hemerocallis sp.	-	-	-	-
Lythrum salicaria	-	(+)	-	(+)
Lactuca sativa	(+)	(+)	(+)	(+)
Cichorium intybus	-	-	-	-
Asclepias syriaca	-	-	(+)	-
Daucus carota	-	(+)	-	-
Ipomoea batatas	-	-	-	-
Brassica napus	-	-	-	-
Rheum rhaponticum	(+)	-	(+)	-
Zea mays	-	-	-	-

2.3.7 Discussion

The range of plant species accepted for adult feeding is wider than that of species suitable for completion of larval development. Even in the presence of field host plants, adult beetles nibbled and fed on non-host plants. Adult feeding and prolongued longevity occurred on spurges, such as *E. nutans*, *E. maculata* (subgenus Chamaesyce) and *E. milii* (subgenus Euphorbium), that did not support larval development to adult. Moderate adult feeding, prolongued longevity and some larval development occurred on species in subgenus Agaloma. The beetles on *E. incisa* (subgenus Esula) were long lived, but only very few larvae survived on this species. In contrast, as for *A. ovata* and *A. venustula*, *E. lathyris* supported larval development but not normal adult longevity. This indicates that the factors supporting adult and larval survival are different. Substantial adult feeding was restricted to genus *Euphorbia*, but slight feeding occurred on most species in family Euphorbiaceae. Slight and prolongued feeding and longevity in no-choice tests occurred on a number of "unsual" species like *Lactuca sativa*, *Daucus carota*, *Brassica napus*, *Rosa* sp. and *Prunus* sp. Also, some nibbling occurred on most plant species tested, which indicates a strong behaviour to chew almost any plants for host recognition.
The results of larval development tests show that larvae can develop on most, if not all, spurge species in subgenus Esula. Some larval development to adults was recorded on species in subgenus Agaloma, and one larva developed to adult on *E. milii* (subgenus Euphorbium).
The experimental host range of *A. violacea* appears to be slightly broader than that of *A. venustula* and *A. ovata*, with substantial adult feeding and longevity on several spurge species in four of the five subgenera tested (exception is subgenus Poinsettia). This species also shows a pronounced behaviour by the adult to chew for host recognition resulting in prolongued longevity in absence or low concentration of a severe deterrent in the plant chewed.

2.4 General discussion about *A. venustula*, *A. ovata* and *A. violacea*

Aphthona venustula, *A. ovata* and *A. violacea* are the best suited European *Aphthona* species for biocontrol of leafy spurge in shaded and high moisture habitats. Both *A. venustula* and *A. ovata* are species of mesic and submesic forests and transitional areas for which there are currently no species available in North America. *Aphthona venustula* has a broad macroclimatic range within its European distribution range being found from the Mediterranean area, to Central and northern Europe. It is thus adapted to a wide variety of climates, ranging from Mediterranean to subantlantic, and subcontinental. *Aphthona ovata* is best adapted to subcontinental and mountain areas in Europe. It should spread into areas unsuitable for *A. venustula*. *Aphthona violacea* is a continental species adapted to humid habitats.
The three species have in common that they overwinter as adults. *Aphthona venustula* and *A. ovata* are univoltine whereas *A. violacea* has probably a partial second generation. For all three species, normal adult feeding and longevity in no-choice tests occurred on nearly all spurges in subgenus Esula. Adult feeding and prolongued longevity occurred on some spurge species in subgenera Agaloma, Chamaesyce, Euphorbium, and occasionally Poinsettia. Slight adult feeding and little prolongued longevity occurred on some species in family Euphorbiaceae as well, in particular with *A. violacea*. Slight adult feeding, usually without prolongued longevity, was observed with *A. venustula* and *A. ovata* on species like *Geranium* sp., *Rosa* sp., *Prunus* sp., *Lythrum salicaria*, *Lactuca sativa*, *Brassica napus* and *Rheum rhaponticum*. With *A. violacea*, prolongued longevity and extended feeding was recorded on a number of species, like *Geranium* sp, *Rosa* sp., *L. sativa*, *Cichorium intybus*, *Daucus carota* and *Ipomoea batatas*. This

species shows a pronounced behaviour by the adult to chew for host recognition resulting in prolongued longevity in absence or in case of low concentration of a deterrent in the plant chewed. However, the potential adult host range of *A. violacea* under field conditions should be much narrower because of its habitat specialisation.
Even in the presence of field host plants, adult beetles of all three species nibbled and fed on non-host plants outside genus *Euphorbia* and/or family Euphorbiaceae.

For all three species, the range of plant species accepted for adult feeding is considerably wider than that of species suitable for completion of larval development. All species in subgenus Esula are to some degree suitable host plants for larval development. Larvae can develop successfully on species in subgenus Agaloma (e.g. *E. discoidalis*, *E. marginata*, and in particular *E. corollata*), and rarely in subgenera Euphorbium (*E. milii*, *E. tirucalli*) and Poinsettia (*E. heterophylla*) (Table 11). No larval development to adult was observed in subgenus Chamaesyce, and outside genus *Euphorbia*. All species tested in subgenus Chamaesyce are annual species. It should be noted also that there is no species under review for legal protection in subgenus Agaloma.

Given the results obtained so far with these three *Apththona* species, it is recommended to make additional tests with, 1) new species in subgenus Agaloma like *E. hexagona* (annual, sympatric with leafy spurge), *E. nephradania* (annual, sympatric with leafy spurge), *E. strictior* (perennial, possibly sympatric with leafy spurge), *E. zinniiflora* (perennial, sympatric with leafy spurge in eastern USA), *E. ipecacuanhae* (perennial, sympatric with leafy spurge in eastern USA), or *E. exserta* (perennial), 2) to test perennial species in subgenus Chamaesyce like *E. fendleri*, *E. polycarpa* (both sympatric with leafy spurge) or *E. deltoidea* which is under review for legal protection, and 3) to test endangered or threatened species in subgenus Esula, i.e. *E. purpurea* and *E. telephiodes*. *Euphorbia purpurea* is of particular importance since it is sympatric with leafy spurge in eastern USA and it occurs in moist habitats.

Table 11: Plant species sustaining larval development to adult of *A. venustula*, *A. ovata* and *A. violacea*

	A. venustula	*A. ovata*	*A. violacea*
Euphorbiaceae			
Genus *Euphorbia*			
Subg. **Chamaesyce**			
* *E. maculata*	---	---	---
* *E. nutans*	---	---	---
* *E. prostrata*	---	---	---
E. chamaesyce		---	---
* *E. glytosperma*		---	
Subg. **Agaloma**			
* *E. marginata*	---	rare ?	rare ?
* *E. corollata*	yes	yes	yes
* *E. discoidalis*	rare	---	
* *E. antisyphilitica*	---	---	
Subg. **Poinsettia**			
E. pulcherrima	---	---	---
* *E. heterophylla*	---	rare	---
* *E. cyathophora*	---	---	---
Subg. **Euphorbium**			
E. tirucalli	---	rare	---
E. milii	rare	---	rare
Subg. **Esula**			
E. lathyris	yes	yes	yes
E. polychroma	yes	yes	yes
E. helioscopa	yes	yes	yes
E. characias	yes	yes	yes
E. peplus	yes	yes	yes
* *E. spatulata*	yes	yes	yes
* *E. incisa*	yes	yes	yes
E. cyparissias	yes	yes	yes
E. seguieriana	yes	yes	yes
E. amygdaloides	yes	yes	yes
N-A- Leafy spurge	yes	yes	yes
E. salicifolia	yes	yes	yes
E. myrsinites	yes	yes	yes
Acalypha hispida	---	---	---
Ricinus communis	---	---	---
Mercurialis perennis	---	---	---
Pedilanthus tithymal.	---	---	---
Croton variegatum	---	---	---
Manihot esculenta	---	---	---
Plants attacked by other species in genus *Aphthona*	---	---	---
Plants with latex in other families	---	---	---
Economic plants	---	---	---

2.5 *Chamaesphecia* sp. from China

Since the larvae of *C. schroederii* sp.n. collected from *E. linifolia* (?) in Inner Mongolia did not develop in the roots of North American leafy spurge, another attempt was made to select one *Chamaesphecia* species from China for biological control of leafy spurge. From 1993 to 1995, six shipments of roots of *E. pekinensis* (?) infested by *Chamaesphecia* sp. were received from Inner Mongolia at various periods of time. The attack rate for most of these collections turned out to be very low, or the larvae died during the transport. In 1994 only, some 60 larvae were transferred to 12 potted North American leafy spurge, but no larval development was recorded on the target weed. The moth was determined by I. Tosevski as closed to *C. tenthrediniformis*.

2.6 *Oberea doncelii* from China

2.6.1 Taxonomy

The beetle previously believed to be *O. erythrocephala*, was identified as *O. donceeli* var. by C.L. Vaamonde, Museo de Historia Natural "Luis Iglesias", Santagio de Compostela, Spain.

2.6.2 Host range and discussion

The first 32 adults of *O. donceeli* emerged in 1993 from potted leafy spurge plants to which some 90 young larvae received from Inner Mongolia had been transferred in 1992. The larvae had been collected from *E. pekinensis* (?) in Inner Mongolia. Only very few eggs were laid on the control plants in 1993, and the tests were restarted the following year. A total of 71 adults emerged in June 1994 from the 170 larvae (i.e. 42%) received from China and transferred to potted *E. virgata* and North American leafy spurge in 1993. The beetles were tentatively sexed, using the length of the antennae, and used in multiple-choice oviposition tests. Four replicates of two pairs of *O. donceeli* each were put into cages with four randomly selected test plants, and leafy spurge as control. The test plants were: *E. corollata*, *E. pulcherrima*, *E. tirucalli*, *E. milii*, *E. lathyris*, *E. incisa*, *Ricinus communis* and *Manihot esculenta*. In 1995, A total of 49 adults emerged between 5 May and 24 June of a total 65 larvae (i.e. 75%) which had been transferred onto potted European and North American leafy spurge in 1994. The newly emerged beetles were kept in plastic cylinders for mating and sexing. Twenty one females have been observed in copulation and were used in the same designed multiple oviposition tests. No-choice oviposition and larval development tests were made with *E. tirucalli*, *E. corollata* and *E. pulcherrima* accounting for six female-days each. In 1996, a total of 87 adults emerged from the 250 larvae (i.e. 35%) which had been transferred onto potted European and North American leafy spurge in 1995. Some 35 females have been observed in copulation and were used in multiple-choice oviposition tests. Four replicates of two females of *O. donceeli* each were put into cages with four randomly placed test plants, and North American leafy spurge as control. The cages were checked twice a week, and the control plants replaced. The test plants were replaced at least once during the tests which were run for two to three weeks. After eight to ten days all females were exposed again to males in mating containers. The majority of females mated a second time, and were returned to oviposition cages. All test plants were dissected during summer, i.e. about two months after oviposition, to check for eggs and larvae. A synopsis of all multiple-choice oviposition and larval development tests is given in Table 12.

Table 12 : Synopsis of multiple choice tests with *Oberea donceeli* (1994-1995/1996)

Test Plant	Adult feeding	# eggs/ replicate (SE)	# replicates	% larval survival
Subgenus : **ESULA**				
N-A leafy spurge	++	3.1 (0.6)	12	45.9
N-A leafy spurge 1996	++	4.5 (0.9)	16	52.8
E. incisa	++	3.0 (1.2)	4	16.7
E. lathyris	+	0.0	4	
E. lathyris **1996**	++	0.8 (0.5)	4	66.7
E. polychroma **1996**	+	3.8 (0.9)	4	73.3
E. myrsinites **1996**	(+)	0.0	4	
E. platyphyllos 1**996**	++	4.3 (1.0)	4	47.0
Subgenus **AGALOMA**				
E. corollata	++	2.0 (1.4)	4	0.0
E. corollata **1996**	+	2.0 (1.0)	4	0.0
E. marginata **1996**	++	1.3 (0.6)	4	100.0
Subgenus **POINSETTIA**				
E. pulcherrima	+	0.0	4	
E. heterophylla	+	1.3 (1.3)	4	0.0
Subgenus **EUPHORBIUM**				
E. tirucalli	+	1.8 (0.9)	4	28.6
E. milii	(+)	0.0	4	
Ricinus communis	-	0.0	4	
Mercurialis perennis **1996**	-	0.0	4	
Manihot esculenta	-	0.0	4	
Acalypha hispida	-	0.0	4	
Pedilanthus tithymaloides	-	0.0	4	
Croton sp.	-	0.0	4	
Asclepias syriaca **1996**	+	0.0	4	
Ficus elastica **1996**	-	0.0	4	
Lythrum salicaria **1996**	-	0.0	4	
Pelargonium sp. **1996**	-	0.0	4	
Chrysanthemum leuc. **1996**	-	0.0	4	
Rubus sp. **1996**	-	0.0	4	
Vinca rosea **1996**	-	0.0		
Linum flavum **1996**	-	0.0		
Helianthus annuus **1996**	-	.0.0		

- no feeding
(+) occasional or slight feeding
+ moderate feeding
++ heavy feeding

Moderate to heavy adult feeding was observed on North American leafy spurge, and on most spurge species in subgenus Esula and Agaloma, i.e., *E. lathyris*, *E. platyphyllos*, *E. polychroma, E. incisa*, *E. corollata*, and *E. marginata*. Moderate feeding was recorded on *E. tirucalli*, *E. pulcherrima*, *E. heterophylla*, and outside family Euphorbiaceae, on *Asclepias syriaca* (Table 12). Oviposition and larval development occurred on all species in subgenus Esula (except *E.*

myrsinites). Although oviposition occurs only occasionally on species outside subgenus Esula, several species outside this subgenus may well support larval development (e.g. *E. marginata*) and to a lesser extend *E. tirucalli*.
In no-choice oviposition tests, one living third instar larva was recorded on *E. tirucalli*. Eight dead first instar larvae were found on *E. corollata* within mines of 0.5-2.5 cm in length. No eggs were found on *E. pulcherrima*. These results confirmed those obtained in multiple-choice oviposition tests.
Within subgenus Esula, it is unclear to which extend leafy spurge is a preferred host for *O. donceeli*. It does not seems that *O. donceeli* shows great promise for the biological control of leafy spurge although it might extend its distribution slightly further north into the Canadian prairie where *O. erythrocephala* does not occur.

2.7 *Obera moravica*

2.7.1 Taxonomy

O. moravica sp. n. has been described recently by Kratochvil (1989). The holotype is from Moravia (Czech Republic). It is about 11.0-14.0 mm in length. *O. moravica* is very similar to *O. euphorbiae*, which is a larger and more robust species reared from *E. palustris*.

2.7.2 Host plants and geographical distribution

Host plant records of *O. moravica* are controversial. Kratochvil (1989) mentioned *E. lucida* and *E. palustris* as host plants. According to Adelbauer (1994), the beetle is found only on E. *lucida*. It is known from wet habitats in Central and Eastern Europe.

2.7.3 Host range and discussion

Oberea moravica was collected exclusively from *E. lucida*. In 1995, approximatively 90 and 50 shoots of *E. lucida* infested with mature larvae/pupae of *O. moravica* were collected in April in Serbia and Hungary, respectively. A total of 54 adults have emerged between early May and the end of July. Rate of parasitism by a tachinid and braconid species reached 50 %. In 1996, approximatively 150 shoots of *E. lucida* infested with mature larvae/pupae of *O. moravica* were collected in April in Serbia. A total of 50 adults emerged between mid May and mid July. Rate of parasitism by a tachinid and braconid species reached 30 % that year.

The newly emerged beetles were kept in plastic cylinders for mating and sexing. The observation of mating couples appears to be the safest method for separating males and females of *Oberea* species. The mated females were used in multiple-choice oviposition and larval development tests. The males which had copulated were marked with a small colour dot on the elytra. Some 20 females were observed in copulation and used in screening tests each in 1995 and 1996. Males are able to copulate with more than one female within one day.

Four replicates of two females of *O. moravica* each were put into multiple-choice oviposition cages with four randomly placed test plants. *E. lucida* and North American leafy spurge were used as controls each in two of the four replicates. The cages were checked twice a week, and the control plants replaced. The test plants were replaced at least once during the tests which were run for two to three weeks. After eight to ten days all females were exposed again to males in mating containers. The majority of females mated a second time, and were returned into oviposition cages. All test plants used in oviposition tests have been dissected during summer, i.e. about two months after oviposition, to check for eggs and larvae. The number of eggs laid on *E. lucida* and other attacked species could hardly be recorded because of heavy mining by larvae at the time of dissection. Thus, the number of eggs per replicate (Table 13) expressed in fact the total number of living larvae, dead larvae as well as the number of empty mines and eggs found per replicate. These numbers must be considered with some caution. Oviposition holes with no eggs found were not considered.

Substantial to moderate adult feeding was observed on *E. corollata*, *E. marginata* and *E. milii* (Table 13). Only occasional nibbling occurred on *E. heterophylla* and *E. pulcherrima*. No adult feeding was observed on *E. tirucalli*, *R. communis*, *M. esculenta*, *Acalypha hispida* and *Croton* sp.. Nearly eight eggs per replicate were recorded on *E. lucida*, and 40% less attack was observed on North American leafy spurge. The number of eggs laid was much lower on *E. platyphyllos*, *E. corollata*, *E. marginata*, and *Ricinus communis*. With the exception of *E. corollata*, host suitability for larval development was high for all species which were accepted for oviposition, i.e. *E. lucida*, *E. platyphyllos* and *E. marginata*, and to a lesser extend North American leafy spurge. In addition to two unhatched eggs, one dead first instar larva was found within a mine of 5 cm in length on *R. communis*.

Heavy to moderate adult feeding occurred on spurge species in subgenera Esula, Agaloma and Euphorbium. Although oviposition occurred only occasionally on species outside subgenus Esula, several species outside the weed subgenus may well support larval development. Within genus *Euphorbia*, it seems that plant species with thick and hollow stems are preferred hosts for larval development. North American leafy spurge appears to be a less preferred host both for oviposition and larval development. Considering the problems in establishment encountered with *O. erythrocephala* in North America and its low impact on the target weed, it does not seems that *O. moravica* shows greater promise for the biological control of leafy spurge in the humid habitats for which it was selected .

Table 13 : Synopsis of multiple choice tests with *Oberea moravica* (1995-1996)

Test Plant	**Adult feeding**	**# eggs/ replicate (SE)**	**# replicates**	**% larval survival**
Subgenus : **ESULA**				
E. lucida	++	7.8 (1.8)	11	64.8
N-A leafy spurge	++	4.3 (1.1)	8	35.3
E. seguieriana	+	0.0	4	
E. myrsinites	-	0.0	3	
E. lathyris	(+)	0.0	3	
E. platyphyllos	+	0.5 (0.5)	4	50.0
Subgenus **AGALOMA**				
E. corollata	++	0.9 (0.6)	8	0.0
E. marginata	+	0.5 (0.5)	4	50.0
Subgenus **POINSETTIA**				
E. pulcherrima	(+)	0.0	4	
E. heterophylla	(+)	0.0	4	
Subgenus **EUPHORBIUM**				
E. tirucalli	+	0.0	4	
E. milii	-	0.0	4	
Ricinus communis	-	0.3 (0.3)	4	0.0
Manihot esculenta	-	0.0	3	
Acalypha hispida	-	0.0	4	
Croton sp.	-	0.0	3	

- no feeding
(+) occasional or slight feeding
\+ moderate feeding
++ heavy feeding

3. Conclusions

Following the forthcoming success of the *Aphthona* species in the mid 1980s the decision was taken to center a strategy on this group of agents. Because leafy spurge occurs in Canada in a wide range of habitat types, it was also decided to select agents suited for the four main leafy spurge habitats which have been defined as dry open, mesic open, moist and shaded habitats.

In 1990 releases of *A. lacertosa*, a species adapted to mesic-moist habitats, have started in Canada. In 1991, investigations started on *A. venustula* and *A. ovata*, two species from shaded habitats. Although most of the work was terminated in 1994 and 1995 respectively, screening tests have not yet been fully completed because of an increasing concern over potential feeding on native "threatened and endangered" *Euphorbia* species, and the lack of native spurge species for host specificity studies. In 1995, work started on *A. violacea*, a continental species exclusively associated with humid habitats. This work has not yet been completed for the same reason.

For all three species, the range of plant species accepted for adult feeding is considerably wider than that of species suitable for completion of larval development. All species in subgenus Esula are to some degree suitable host plants for larval development. Larvae can develop successfully on species in subgenus Agaloma (e.g. *E. discoidalis*, *E. marginata*, and in particular *E. corollata*), and rarely in subgenera Euphorbium (*E. milii*, *E. tirucalli*) and Poinsettia (*E. heterophylla*).

Given the results obtained so far with these three *Apththona* species, it is recommended to make additional tests with, 1) other species in subgenus Agaloma like *E. hexagona* (annual, sympatric with leafy spurge), *E. nephradania* (annual, sympatric with leafy spurge), *E. strictior* (perennial, possibly sympatric with leafy spurge), *E. zinniiflora* (perennial, sympatric with leafy spurge in eastern USA), *E. ipecacuanhae* (perennial, sympatric with leafy spurge in eastern USA), or *E. exserta* (perennial), 2) to test perennial species in subgenus Chamaesyce like *E. fendleri*, *E. polycarpa* (both sympatric with leafy spurge) or *E. deltoidea* which is under review for legal protection, and 3) to test endangered or threatened species in subgenus Esula, i.e. *E. purpurea* and *E. telephiodes*. *Euphorbia purpurea* is of particular importance since it is sympatric with leafy spurge in eastern USA and it occurs in moist habitats. In case these species may be to difficult to obtain, they could be replaced by more common ones in subgenus Esula like *E. inundata* or *E. robusta*. Foreign exploration on potential biological control agents of leafy spurge has been interrupted in 1999. Completion of the screening tests of the above mentionned *Aphthona* species will be hopefully completed in 2000 when the native spurge will be available.

In biocontrol of leafy spurge, the root-feeders will be supplemented with insects, such as species in genera *Oberea* and *Pegomya* which damage the shoot and prevent flowering, thus reducing the long distance dispersal of the weed. In 1995-96 work was carried out on *Oberea moravica* sp.n. collected on *E. lucida* in humid sites. Although all food niches and habitats may be filled with European insects, some species, such as *O. erythrocephala*, failed to establish or to build up high population densities possibly due to a lack of climatic matching, and some others, such as *C. hungarica* are expected to do so because they are not fully compatible with Canadian leafy spurge. This is the reason why *O. donceeli* and *Chamaesphecia* spp. from Northern China were studied with the expectation that east-Palearctic spurge insects will be better pre-adapted to leafy spurge and to the northern distribution of the weed. From 1993 to 1995, another *Chamaesphecia* species – closed to *C. tenthrediniformis*- was tentatively collected in China and studied. Between 1994-96, host range studies was carried out with *O. donceeli* from Inner Mongolia on 27 plant species.

The main need is for species adapted to forested and spring flood sites, and those at the northern limit of the spurge range. With this regard, it is unfortunate that neither *O. doncelii*, nor *O. moravica* are specific enough for introduction into North American, and that *C. hungarica* has proven to be a difficult species to establish. In addition, none of the two *Chamaesphecia* species collected in Inner Mongolia, *C. schroederii* and *C*. near *tenthrediniformis* did accept North American leafy spurge as host plants.

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