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Petition for the release of *Aphthona czwalinae* Weise against leafy spurge (*Euphorbia esula*) in the United States

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Part I

TO: Phil Lima, Biological Control Information Staff APHIS

FROM: Robert W. Pemberton

Enclosed are the results of the research on the flea beetle *Aphthona czwalinae* (Chrysomelidae). This petition is composed of two parts. The first is the report "*Aphthona czwalinae* Weise (Coleoptera: chrysomelidae): A candidate for the biological control of leafy spurge in North America" by A. Gassmann which the Working Group reviewed on Canada's behalf. Copies of this report should be in the Working Group's files. This research, which was done at the Commonwealth Institute of Biological Control's Delémont, Switzerland lab, showed *A. czwalinae* to be specific to the genus *Euphorbia* of the Euphorbiaceae. This result agrees with the literature and field records for *A. czwalinae*, which record it from: *Euphorbia esula, E. cyparissias* and *E. virgata.* The Gassmann report also included information on *A. czwalinae's* taxonomic position, life history, laboratory biology, mortality factors, feeding effects on the host plants, as well as a brief description of the target plant – leafy spurge (*Euphorbia esula*), a serious pest of the rangelands of the Great Plains of North America. Based on this research, the Working Group gave permission to release *A. czwalinae* in Canada and to import it into the USDA's Biological Control of Weeds Quarantine in Albany, California, for additional testing.

Part II of this petition reports the results of the host specificity testing in Albany. The testing at Albany was undertaken to try to predict what the potential host plant range within the genus *Euphorbia* could be if *A. czwalinae* were released in the United States. The laboratory oviposition, adult feeding and adult longevity tests against 10 representative *Euphorbia* species indicated that *A. czwalinae* is probably restricted to species within the subgenus *Esula*. This suggests that fewer than 19 of the 112 native North American spurges could become possible host plants. Of the 19 subgenus *esula* species which are possible host plants, only 5 species (excluding *E. purpurea*) are sympatric with leafy spurge. Neither of the subgenus *Esula* species (*E. telephioides* and *E. purpurea*), which are under review for endangered status, supported larval development, although there was feeding and oviposition by one female on *E. purpurea*.

A. czwalinae's level of host specificity appears to be similar but more narrow than that of *A. cyparassiae* and *A. flava*, which were cleared for introduction in the United States in 1985 and 1986. The host range seems to be broad enough for it to accept the various forms and hybrids of leafy spurge and yet narrow enough to exclude use of all but a few of the numerous spurges which are native to North America. Both the adults and the larvae damage the host plants, the adults feeding on the leaves and the larvae in the roots. The main effect of *A. czwalinae* feeding on leafy spurge will probably be water stress through reduction of absorptive roots.

If permission is granted for the introduction of *A. czwalinae* in the United States, releases will begin in the 1987 summer season.

If you or other members of the Working Group have questions about *Aphthona czwalinae*, please feel free to call me or Lloyd Andres at FTS 449-3991 or Commercial (415) 486-3757.

Part II

Host plant specificity testing of *Aphthona czwalinae* Weise (Chrysomelidae) against *Euphorbia* species which are native to North America

By Robert Pemberton and Gerald Johnson, USDA Albany Biological Control of Weeds Group.

Introduction

The purpose of the host plant specificity testing in Albany was to study and hopefully, predict what the host range of *Aphthona czwalinae*, within the genus *Euphorbia*, could become if this flea beetle is released in the United States. This was desirable because there are 111-112 spurges (107 *Euphorbia* and 4-5 *Chamaesyce* spp.) native to America north of Mexico. Among these are 9 rare species, which are under review by the U.S. Dept. of the Interior for listing as legally protected, endangered or threatened species. Two Florida species of *Euphorbia* (*E. garberi* and *E. deltoides*) in the subgenus *Chamaesyce* are federally protected endangered and threatened species.

The *Euphorbia* floras of North America and Europe, to which *A. czwalinae* is native, are somewhat different. In Europe all but 4 of 105 native *Euphorbia* belong to the subgenus *Esula*, the group to which leafy spurge belongs. The other four species are members of the subgenus *Chamaesyce*. In addition to the subgenus *Esula* with 21 species, North America has the subgenera *Agaloma* with 26 species, Poinsettia with 3 spp., and *Chamaesyce* with 57 species. The host records for *A. czwalinae* and the other *Euphorbia* feeding insects from Europe are from host plants belonging to the subgenus *Esula* and therefore provide no indication of whether the North American Poinsettia, *Agaloma* and *Chamaesyce* spurges could become hosts. The subgenera of the genus *Euphorbia* are not only useful ways of dealing with the large number of species in the genus, but also appear to be quite natural groupings, which reflect true phylogenies within the genus. Many of the *Euphorbia* feeding insects respond to these subgenera, accepting as host plants most

of the species in one or more of the subgenera and rejecting the species in other subgenera. These subgenera were the basis for selecting test plants and for providing the conceptual framework to judge the host plant specificity of *A. czwalinae* and other candidate insects.

A. czwalinae was brought into quarantine at the USDA's Albany laboratory on the basis of the host specificity testing done at the Delémont Switzerland laboratory of the Commonwealth Institute of Biological Control by Gassmann. This work showed the flea beetle to be specific to the genus *Euphorbia*. Table 5 summarizes the results of the testing of *Euphorbia* species by Delémont. *Euphorbia maculata, E. marginata, E. corollata,* and *E. heterophylla* are U.S. spurges, which were tested in Delémont.

Table 1 shows the plants, which were used in the host plant specificity testing of *A. czwalinae* at Albany. The plants were selected to represent the four different subgenera (*Agaloma*, *Chamaesyce*, *Esula*, and *Poinsettia*) of the genus *Euphorbia* which occur in North America. Most of the species (7 of 10) are sympatric with leafy spurge, and 6 of the 10 species could act as bridges to rare protected and review species. *Euphorbia purpurea* and *E. telephioides* are test plants because they are under review and are closely related to leafy spurge (subgenus *Esula* species). *E. hooveri* is a Chamaesyce species under review for protected status. *Euphorbia maculata,* and *E. heterophylla* L. are test plants because they are at times reported as weedy. *Euphorbia marginata* (snow on the mountain), *E. corollata* (flowering spurge), and *E. heterophylla* (fire plant) are cultivated as ornamentals. An effort has been made to select native species that possess a number of the desired characteristics in order to reduce the number of test species needed.

Table 1. Native euphorbias used in host specificity testing for *Aphthona czwalinae.*

	Habit	Subgenus	Sympatric with leafy spurge	Potential bridge	Rare Review species	Weed	Ornamental
Euphorbia heterophylla	Ann.	Poinsettia	X	X		X	X
E. maculata	Ann.	Chamaesyce	X	X		X	
E. serphyllifolia	Ann.	Chamaesyce	X	X			
E. hooveri	Ann.	Chamaesyce			X		
E. robusta	Peren.	Esula section esula	X	X			
E. purpurea	Peren.	Esula section not placed	X		X		
E. telephioides	Peren.	Esula section ipeccacuahae			X		
E. incisa	Peren.	Esula section esula	possibly				
E. marginata	Ann.	Agaloma	X	X		X	
E. corollata	Peren.	Agaloma	X	X		X	

The test plants were grown in 9 cm. fiber pots, from seeds, cuttings and root pieces. The soil used was "UC mix" modified to match the edaphic conditions of the specific tests species. *Euphorbia purpurea, E. telephioides,* and *E. robusta* proved difficult to obtain and slow growing. The adult *A. czwalinae* used in the testing, were obtained from *Euphorbia esula* growing in Austria near Vienna.

Procedures

The host specificity testing strategy was first to do a combination adult feeding, adult longevity and oviposition tests with all of the test species. If oviposition or significant longevity occurred on *Euphorbia* species outside the subgenus *Esula* or on the rare *E. telephioides* or *E. purpurea* within the subgenus *Esula*, then larval transfer tests would be done on those species to see if they could support development.

Ten individuals of each test plant species were used in the testing. A male and a female of *A. czwalinae* were placed inside a 15-dram ventilated plastic cylinder, which enclosed a leafy branch of the test plant growing in a pot. A moist paper towel was wrapped around the base of the enclosed branch within the cylinder (Fig. 1-experimental setup). *A. czwalinae,* as with *A. flava* and *A. cyparissiae*, normally oviposits in the soil around the base of its host plant. These species will readily lay eggs between the paper toweling of the stems of acceptable host plants. Periodic removal and examination of the toweling allows the number of eggs to be readily determined. The number of eggs laid in soil at the stem bases of plants can be difficult to accurately determine. The number of eggs laid per test species and the percentages of individual test plants receiving eggs were recorded. The presence or absence of adult feeding and the number of adults living through time (1 week, 2 weeks, and 1, 2, 3 and 4 months) was recorded for each test plant.

Since there was some oviposition on the rare review species *Euphorbia purpurea*, larval transfer tests were conducted to determine whether larval development could occur on this species. Development of larvae from 1st instar to mature 3rd instar (the last larval stage) has become the standard method of examining development of *Aphthona* flea beetle for the Delémont and Albany laboratories. This method is used because the high mortality of overwintering 3rd instar larvae in laboratory cultures prevents an accurate evaluation of full development (from 1st instar larvae to adult). The 1st to 3rd instar development test overestimates an insect's acceptance of test plants, since the ability to form the pupal and adult stages is not examined.

Twenty newly hatched larvae, laid by *A. czwalinae*, that had fed in *E. esula* were transferred to 15 *E. purpurea* and 15 *E. esula* (Montana) control plants growing in 15 cm fiber pots. The larvae were placed on the moist rhizomes of the test plants that were growing near the soil surface. The *E. purpurea* primary rhizome averaged 1 cm in diameter and 15 cm in length.

Two and one half to three months after the larvae were transferred (sufficient time for development to the third instar stage), the rhizomes were dissected and the soil was searched for larvae. The larval transfer tests were done from August 1986 to January 1987. During this time, the quarantine test environment temperature ranged from a high 21º C during the day to a low of 10º C at night. The tests were conducted under natural light conditions.

Table 2. Adult longevity, feeding and oviposition at Albany 1986.

Euphorbia species[1] (subgenus)	Number of Adults Living Through Time[2.]												Number of plants with		X number of eggs
	1 week		2 weeks		1 month		2 months		3 months		4 months		adult		
	females	males	females	males	females	males	female	male	female	male	female	male	feeding	eggs	per plant
E. esula (esula)	10	10	6	9	4	8	3	3		1		1	10	9	62.4
E. robuta (esula)	10	9	9	4	9	4	6	3	2		1		10	9	129.3
E. incisa (esula)	8	9	7	7	7	6	4	4		1			10	9	91.8
E. purpurea (esula)	2		2		1		1						4	1	7.6
E. telephiodes (esula)	1														
E. marginata (agaloma)	2		1										1		
E. corollata (agaloma)															
E. pulcherima (poinsettia)	1												1		
E. heterophylla (poinsettia)															
E. serphyllifolia (chamaesyce)															
E. hooveri (chamaesyce)							1								
E. maculata (chamaesyce)															

[1.] Ton plants per species of plant.

[2.] 20 adults (10 ♀ and l0 ♂) were tested test species

Results and discussion

Table 3 shows the results of the adult longevity, feeding and oviposition tests. A majority of the adult beetles lived for more than one month and a few to three months on *E. esula, E. robusta* and *E. incisa*. A single female lived for two months on *E. purpurea*. No adults lived for more than a few weeks, and usually less than one week, on test plants belonging to subgenera other than *Esula*. Adult feeding and oviposition followed the same pattern. All 10 test plants of *E. esula, E. robusta* and *E. incisa* had adult feeding, while 9 of 10 plants these species supported oviposition. One *E. purpurea* plant supported oviposition and 4 plants had adult feeding. Plants outside the subgenus Esula had no adult feeding or oviposition except for nibbling one plant of *E. marginata* and *E. pulcherima*. The large numbers of eggs (X 62.4-129.3) laid on the favored plants indicated that the beetles were behaving normally within the confines of the plastic cylinders.

Table 3. Summary of *Aphthona czwalinae* host plant specificity testing on native North American *Euphorbia* species, Albany 1986, and the target weed -*E. esula virgata.*

Test Plant Species	Subgenus	% of plants with adult-feeding	% of plants supporting oviposition	% of adults living 1 month or longer
Euphorbia esula	Esula	100	90	60
Euphorbia incisa	Esula	100	90	65
Euphorbia robusta	Esula	100	90	65
Euphorbia purpurea	Esula	40	10	5
Euphorbia telephioides	Esula	0	0	0
Euphorbia maculata	Chamaesyce	0	0	0
Euphorbia serphyllifolia	Chamaesyce	0	0	
Euphorbia hooveri	Chamaesyce	0	0	0
Euphorbia corollata	Agaloma	0	0	0
Euphorbia marginata	Agaloma	10	0	0
Euphorbia heterophylla	Poinsettia	0	0	0

Table 4 shows the results of the larval transfer tests on *E. purpurea* and the control *E. esula*. No larvae were found, nor was feeding damage to the roots detected on *E. purpurea*. Larvae were found in 12 of 15 *E. esula* pots. The larvae, which averaged 3.4 per pot of *E. esula* were all in the third instar and had mean body lengths of 5.7 mm. Larval feeding was detected on the roots of 13 of the 15 *E. esula* plants. Since *E. purpurea* could not support larval development, it was not considered to be an unacceptable host plant for *A. czwalinae*.

Table 4. *Aphthona czwalinae* larval transfer test on *Euphorbia Purpurea*. 20 first instar larval transferred to each plant.

	E. purpurea		*E. esula* (control)	
Plant number	number of larvae found	Feeding damage	number of larvae found*	Feeding damage
1	0	–	10	+
2	0	–	3	+
3	0	–	1	+
4	0	–	4	+
5	0	–	7	+
6	0	–	2	+
7	0	–	3	+
8	0	–	0	-
9	0	–	5	+
10	0	–	2	+
11	0	–	1	+
12	0	–	0	-
13	0	–	7	+
14	0	–	0	+
15	0	–	6	+
		–	$\bar{x}$ = 3.4/pot	

*All the larvae found were third instar.

Table 5 summarizes the results of the *A. czwalinae's* host specificity testing against representative *Euphorbia* species in Delémont and Albany. None of the species outside the subgenus *Esula* supported larval development or oviposition. Within the subgenus *Esula* the rare species – *E. telephioides* and *E. purpurea* were not acceptable host plants.

The subgenus *Esula* contains 21 of the 112 *Euphorbia* species native to America north of Mexico. Two of the 4 species of the subgenus *Esula* were suitable host plants in the laboratory testing. This indicates that only a portion of the 21 species belonging to the subgenus *Esula* would be suitable host plants for *A. czwalinae*. Only six (five excluding *E. purpurea*) are sympatric with leafy spurge. These are: *E. robusta; E. lurida, E. spatulata, E. obtusata,* and *E. crenulata*.

Aphthona czwalinae appears to be a more narrow specialist than *A. flava* and *A. cyparissae*, which were approved for release in the United States in 1985 and 1986. Its host plant specificity appears to be broad enough to attack the various problem forms of leafy spurge and yet narrow enough to exclude the majority of native *Euphorbia* species including the rare and protected species.

A. czwalinae could be a useful addition to the complex of the biological agents under development to try to control leafy spurge. It may establish at more northerly areas than *A. flava* and *A. cyparissinae* or perhaps different sites. In Europe the *Aphthona* feeding on *Euphorbia* species tend to occur in different habitats. Since leafy spurge in North America grows in many different habitats within its large range, it is desirable to use a complex of *Aphthona* species against it.

Table 5. ***Aphthona czwalinae*** **acceptance on species within the genus** ***Euphorbia.***

Test species	Subgenus	Delémont (larval development)	Albany (oviposition)
Euphorbia esula	Esula	+	
• *cyparissias*	•	+	+
• *incisa* (U.S. native)	•		+
• *robusta* (native)	•		+
• *myrsinities.*	•		
• *oblongata*	•		
• *peplus*	•		
• *lathyris*	•		
• *amgdaloides*	•		
• *seguieriana*	•		
• *telephioides* (native)	•		
• *virgata*			
• *purpurea* (native)			+ (no development in larval transfer test)
• *polychroma*	•		
• *maculata* (native)	Chamaesyceae		
• *hooveri* (native)	•		
• *serphylifolia* (native)	•		
• *corollota* (native)	Agaloma		
• *marginata* (native)	•		
• *antisyphilitica*	•		
• *pulcherrima*	Poinsetta		
• *heterophylla* (native)	•		
• *tirucalli*	Euphorbium		
• *milli*	•		

+ = complete larval development (Delémont), adult feeding and oviposition (Albany) failed to complete development
- = failed to complete development

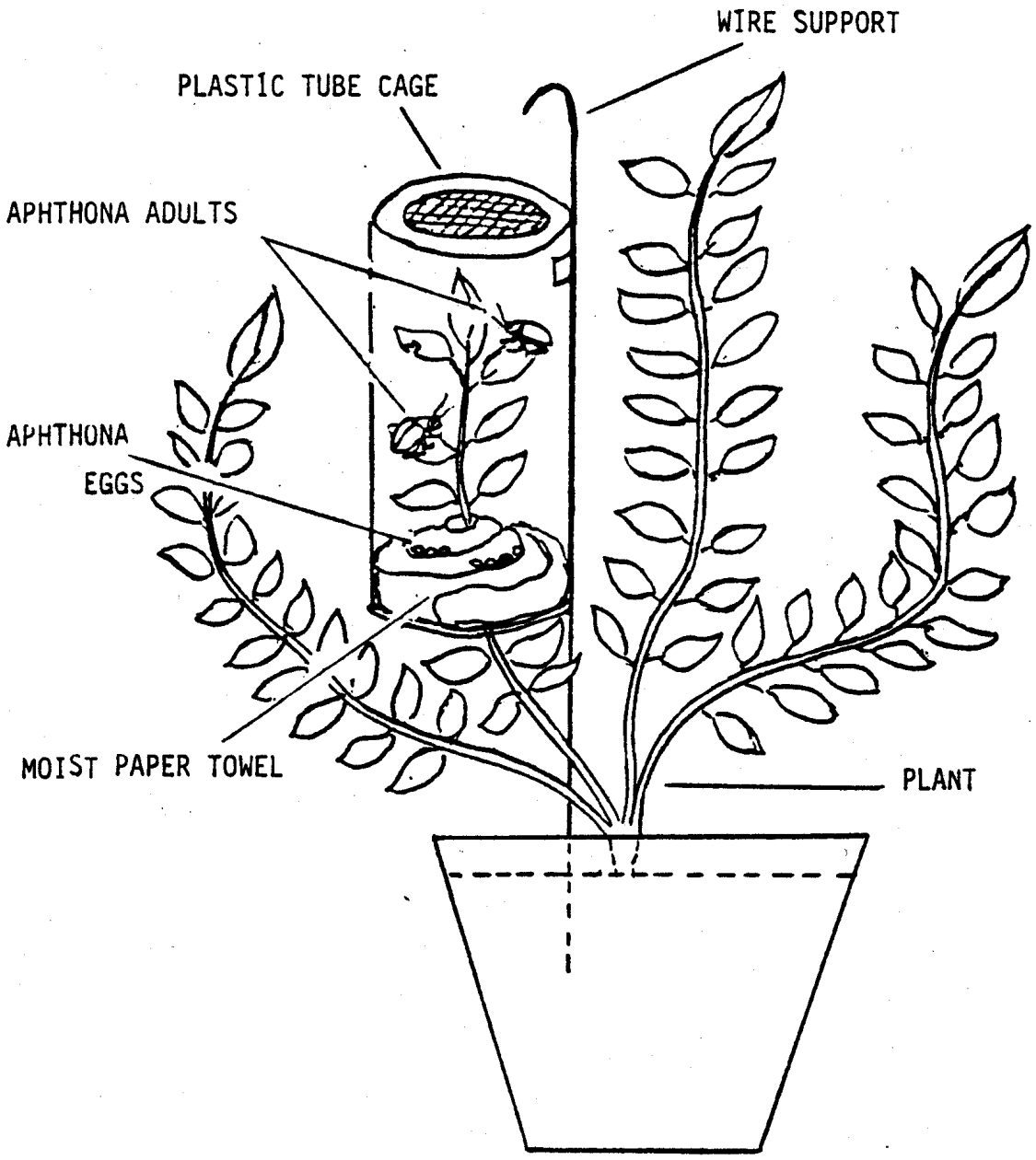

Fig. 1. Oviposition, adult feeding and longevity test set up.