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***Aphthona czwalinae*: Permit application information supplement**

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**Release of nonindigenous biological control agents:
Permit application information supplement**

USDA-APHIS-PPQ-BATS

<p><i>Aphthona czwalinae</i> (Coleoptera: Chrysomelidae)</p>
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1. Proposed action

Field release of the flea beetle, *Aphthona czwalinae* Weise (Coleoptera: Chrysomelidae), for the biological control of the exotic weed, leafy spurge (*Euphorbia esula* L.), in the United States

2. Details of proposed action

2.1 Purpose of the release(s)

Mixed-species releases of *Aphthona czwalinae* and *A. lacertosa* will be used to initiate or augment populations at field insectary sites (FIS) in various states. Once these FIS populations are successfully established and are deemed sufficiently large, *A. czwalinae* and *A. lacertosa* will be collected and distributed to leafy spurge-infested areas throughout the state.

Adult *Aphthona czwalinae* consume leafy spurge foliage, but this damage has little or no impact on the weed. However, *A. czwalinae* larvae feed on spurge roots, and this damage may kill leafy spurge plants. *A. czwalinae* larvae may cause mortality directly, by disrupting water and nutrient transport and storage, or indirectly, by providing entry sites for soil borne pathogenic fungi.

Thus, the primary role of *Aphthona czwalinae* in the leafy spurge biocontrol program is to cause mortality among leafy spurge plants, and to reduce the competitiveness of those plants that do survive. This, in turn, should lead to increased competitiveness and abundance of native and forage plants.

2.2 Need for release

Leafy spurge is a perennial herbaceous plant native to Europe and Asia. Since its accidental introduction beginning in the nineteenth century (Dunn 1985), leafy spurge has become a widespread and economically-important

weed in the northern United States and in Canada. The major economic impact of leafy spurge is based on reduced cattle production on infested rangelands (Leistritz *et al.* 1992). The weed also has an adverse ecological impact by displacing native plants (Belcher and Wilson 1989) and, perhaps, by degrading wildlife habitats (Wallace *et al.* 1992).

A variety of tactics may be employed in managing weedy plants. Chemical (herbicides) and cultural (e.g. sheep grazing, cultivation) control techniques may be effective against leafy spurge in some situations, but their widespread utilization is limited by logistical, economic, or environmental constraints. Biological control, however, may offer an opportunity for large-scale, cost-effective leafy spurge management, especially in remote areas and in low-value grazing lands.

2.3 Specific location of the rearing facility and release site(s)

Living *Aphthona czwalinae* and *A. lacertosa* adults are difficult to separate based on standard morphological characteristics. Initial US releases of *A. czwalinae* involved what were believed to be "pure" collections of this species from Europe. However, later evaluations of these US populations revealed the presence of two species, *A. czwalinae* and *A. lacertosa*. Thus, subsequent redistribution collections from these sites have employed, and will continue to employ, mixed populations of the two flea beetle species.

Aphthona czwalinae and *A. lacertosa* adults will be collected from established field insectary sites in North Dakota and, possibly, Montana. In 1995, these insects will be provided for field release in some or all of the following states: Colorado, Idaho, Iowa, Michigan, Minnesota, Montana, Nebraska, New Mexico, Nevada, North Dakota, Oregon, South Dakota, Utah, Washington, Wisconsin, and Wyoming. Releases may also be made in other states in 1995 and future years.

2.4 Number to be released

Generally, 1,000 to 10,000 *Aphthona czwalinae* and *A. lacertosa* adults will be provided to each state in 1995. The exact number will be determined after assessing the *A. czwalinae/A. lacertosa* populations at the North Dakota and Montana collection sites in summer 1995.

2.5 Timing of release

Apththona czwalinae and *A. lacertosa* adults will be collected in mid- to late July 1995, depending on local weather conditions. Field release in the various states will be accomplished within two days of collection in Montana.

2.6 Methods used for release

Releases of *Apththona czwalinae* and *A. lacertosa* adults will be made at open (uncaged) field insectary sites.

3. Biology of target organism

3.1 Common name, scientific name, and taxonomic classification

Common name: Leafy spurge

Scientific name: *Euphorbia esula* L.

Phylum: *Magnoliophyta* Subtribe: *Euphorbiinae*
Class: *Magnoliopsida* Genus: ***Euphorbia***
Subclass: *Rosidae* Subgenus: *esula*
Order: *Euphorbiales* Section: *esula*
Family: ***Euphorbiaceae*** Subsection: *esulae*
Subfamily: *Euphorbioideae* Species: ***esula***
Tribe: *Euphorbieae*

The taxonomic status of leafy spurge, and the family Euphorbiaceae in general, has not yet been fully resolved. There is a great deal of morphological and biochemical variation among Eurasian and introduced North American populations of *Euphorbia esula* (Crompton *et al.* 1990, Evans *et al.* 1991, Holden and Mahlberg 1992, Manners and Davis 1984). This has led to some classifications of the weed as a complex of numerous species and interspecific hybrids (Ebke and McCarty 1983, Radcliffe-Smith 1985). However, there is ample evidence that leafy spurge can be treated as a single, though highly variable, species (Crompton *et al.* 1990, Evans *et al.* 1991, Harvey *et al.* 1988), a classification that will be followed in this document.

3.2 General life history

Leafy spurge is a long-lived herbaceous perennial plant whose aboveground stems die back each fall but whose well-developed root system and adventitious buds persist from year to year (Best *et al.* 1980). Buds initiate elongation during the fall but remain below the soil surface

throughout the winter (Messersmith *et al.* 1985). Bud elongation resumes in early spring and the aboveground portions of the plant (shoots) become apparent in April or May, depending on location.

Vegetative shoots develop rapidly and reach their full height in June or July. Mature stem heights are variable, depending on soil and weather conditions, but generally range from 0.25 to 1.5 m. Stems may be branched or unbranched. Leafy spurge leaves are usually green or grayish-green in color, linear or lanceolate in shape, and from 2-8 cm long and 2-10 mm wide (Best *et al.* 1980). Leaves are arranged alternately along the stem.

Leafy spurge possesses specialized, unisexual flowers in a compound umbellate arrangement (Selleck *et al.* 1962). On some shoots, flower buds begin to develop several weeks after the shoots first appear, with peak flowering occurring in June and July (Best *et al.* 1980). Flowers may also appear later during the growing season, depending on weather conditions (Raju 1985). Because of the sticky pollen, asynchrony in maturity of adjacent male and female flowers, and the presence of nectaries, leafy spurge appears to be pollinated primarily by insects (Best *et al.* 1980, Messersmith *et al.* 1985). Seeds develop and mature from July through September, and are then explosively expelled up to 5 m from the parent plant; generally, from 30-150 seeds are produced by each flowering shoot (Selleck *et al.* 1962). Leafy spurge seeds may be dispersed over longer distances by flowing water (Selleck *et al.* 1962), birds (Blockstein *et al.* 1987) or grazing mammals (Lacey *et al.* 1992). Spurge seeds are also transported by humans in gravel, soil, hay, or farm equipment (Messersmith *et al.* 1985).

Leafy spurge seeds usually germinate in the early spring (Best *et al.* 1980). Though viability decreases over time, some dormant seeds may germinate after 10 or more years in the soil (Bowes and Thomas 1978, Selleck *et al.* 1962). Typically, spurge seedlings develop a single shoot during the first growing season but do not flower (Messersmith *et al.* 1985). Seedling shoots are usually shorter and less robust than those originating from root buds, reaching heights of 20 cm or less. During the initial growing season, seedlings begin to develop an extensive root system that may extend up to about 1 m and that possesses many root buds (Messersmith *et al.* 1985). In general, seedlings serve to initiate new leafy spurge patches; exposed mineral soil associated with disturbance (e.g. cultivation, trails, roads, overgrazing) seem best suited for leafy spurge

seedling and, hence, patch establishment (Belcher and Wilson 1989). Only a small percentage of newly-germinated seedlings are able to survive in established patches (Best et al. 1980).

Established spurge patches possess a network of lateral and vertical roots that serve perenniating, reproductive, and nutrient and water storage functions (Stroh et al. 1990). Most of the root biomass is located in the upper 15 cm of the soil (Selleck et al. 1962), but some vertical roots may reach depths of 9 m or more (Best et al. 1980). Two types of adventitious buds are formed on the root system (Messersmith et al. 1985): **crown** buds are formed on the root crown, at the base of a current-year shoot, while **root** buds may form almost anywhere along the lateral and vertical roots. Generally, one or more crown buds will elongate at the same location each year, while the number and location of elongating root buds varies greatly from year to year and from plant to plant (Messersmith et al. 1985). Root buds may be formed "spontaneously" or in response to root injury (Messersmith et al. 1985). Most root buds occur near the soil surface, but some may be found more than 3 m deep along vertical roots (Stroh et al. 1990).

Only a subset of crown and root buds formed in a given year actually produce aboveground shoots, through a system of hormonal control and, perhaps, competition for water and nutrients (Raju 1985). Removal of shoots (e.g. mowing or grazing) usually causes activation of some dormant crown and root buds, and the production of new shoots; new shoot density often exceeds that observed before the treatment (Messersmith et al. 1985). Leafy spurge root fragments as small as 2 cm long will produce new shoots and root systems and, hence, new plants, provided they remain buried in soil (Messersmith et al. 1985).

Established leafy spurge plants expand vegetatively, through elongation of the horizontal roots and the formation of adventitious buds (Best et al. 1980). As some of these buds produce shoots in subsequent years, new root crowns are formed. The root connections among the new root crowns and the parent root system eventually disintegrate, resulting in independent, "daughter" plants. Through lateral expansion of the root systems, leafy spurge patches expand up to about 1 m in radius each year (Selleck et al. 1962, Stroh et al. 1990). At a given location, the rate of radial increase in patch size remains fairly constant from year to year (Selleck et al. 1962).

3.3 Pest status

Leafy spurge occurs in at least 30 US states and nine Canadian provinces (Dunn 1979), but is a significant economic problem primarily in Minnesota, Montana, Nebraska, North Dakota, South Dakota, Wyoming, Alberta, Manitoba, and Saskatchewan. Currently, about 660,000 ha are infested in Montana, North Dakota, South Dakota, and Wyoming, and the affected area doubles in size about every 10 years (Leitch *et al.* 1994).

Leafy spurge, like other members of the Euphorbiaceae, contains a milky latex throughout all parts of the plant. This latex is composed of a complex of chemicals, including a variety of terpenoid compounds (Mahlberg 1989). Some latex chemicals from leafy spurge have skin irritant and tumor-inducing properties in mammals (Seip and Hecker 1982, Upadhyay *et al.* 1978). Paradoxically, other compounds from *E. esula* latex may have antileukemic properties; plants in the family Euphorbiaceae have long been used to treat cancers and tumors in traditional medicine (Kupchan *et al.* 1975).

Due to its latex chemistry, leafy spurge can induce a variety of digestive maladies or, in sufficient quantities, may cause death when consumed by cattle (Kronberg *et al.* 1993). However, spurge is rarely eaten by cattle, who instead avoid spurge-infested pasture despite the presence of palatable grasses (Hein and Miller 1992, Lym and Kirby 1987). Significant (>50%) reductions in forage utilization by cattle result when leafy spurge achieves 10% or more of plant cover (Hein and Miller 1992). Interestingly, domestic sheep and goats are able to consume leafy spurge with no visible detrimental effects (Landgraf *et al.* 1984).

Leafy spurge is an aggressive competitor that, because of its expansive root system and dense shoot growth, is able to outcompete rangeland grasses and forbs for available water, nutrients, and light. In addition, leafy spurge appears to exert an allelopathic effect on other plants, possibly through chemicals leached from decomposing leaf, stem, and root tissues (Steenhagen and Zimdahl 1979). Generally, the abundance of grasses and other forbs is significantly reduced in established leafy spurge patches, and some species may disappear altogether (Belcher and Wilson 1989, Nowierski and Harvey 1989).

Leafy spurge infestations significantly reduce the abundance of native prairie plants (Belcher and Wilson 1989). This reduction in native plant diversity may have a

negative impact on wildlife populations (Wallace et al. 1992).

The primary economic impacts of leafy spurge are based on reductions in available forage and, hence, reduced cattle production on infested rangeland. In Montana, North Dakota, South Dakota, and Wyoming, Leitch et al. (1994) estimate that direct and secondary losses due to lost cattle production approach \$120 million a year. An additional \$10 million in non-agricultural (e.g. recreational and watershed) losses (Wallace et al. 1992) bring the total losses due to leafy spurge to about \$130 million annually in the four-state area (Leitch et al. 1994).

4. Biology of organism to be released

4.1 Common name, scientific name, and taxonomic classification

Common name: none

Scientific name: *Aphthona czwalinae* Weise

Phylum: <i>Arthropoda</i>	Superfamily: <i>Chrysomeloidae</i>
Class: <i>Insecta</i>	Family: <i>Chrysomelidae</i>
Subclass: <i>Pterygota</i>	Subfamily: <i>Halticinae</i>
Division: <i>Endopterygota</i>	Tribe: <i>Aphthonini</i>
Order: <i>Coleoptera</i>	Genus: <i>Aphthona</i>
Suborder: <i>Polyphaga</i>	Species: <i>czwalinae</i>

4.2 Taxonomic specialist(s) who identified organism

Mixed *Aphthona czwalinae*/*A. lacertosa* populations at the collection locations (see 2.3, above) originated with insects collected from Europe. These insects were identified by R.E. White, USDA-ARS, Systematic Entomology Laboratory (SEL). Subsequent confirmation of *A. czwalinae* adults from these FIS has been made by Drs. White and A. Konstantinov of the SEL.

4.3 Location of voucher specimens

Type material for *Aphthona czwalinae* is kept at the International Institute of Biological Control in Delémont, Switzerland, the Canadian National Insect Collection in Ottawa, and the US National Museum of Natural History (USNM) in Washington, DC. Voucher specimens from later redistribution collections are kept at the SEL and USDA-APHIS Bozeman Biological Control Facility.

4.4 Natural geographic range of organism

Aphthona czwalinae occurs from central Europe east throughout Russia and into central Asia (Gassman 1984).

4.5 Location where organism was originally collected

Insects initially released in the United States were collected in Austria and Hungary.

4.6 General life history

There is little published information available on *Aphthona czwalinae* biology, but its life history is similar to that of other univoltine *Aphthona* species (Maw 1981, Rees and Spencer 1993). *A. czwalinae* overwinters as a diapausing larva, in the soil and on or near a leafy spurge root. Overwintered larvae resume development in the spring, and pupation occurs within a soil cell from late spring to early summer. Adult beetles emerge from the soil throughout the summer, and begin feeding on leafy spurge leaves and flowering structures. *A. czwalinae* adults are about 3 mm long; they rarely fly under field conditions and instead move about by hopping in typical flea beetle fashion. Adults are relatively long-lived beetles, capable of surviving several weeks to several months, depending on field conditions (Maw 1981). At a given location, *A. czwalinae* adults begin to emerge and reach their peak abundance earlier than do adults of most other introduced *Aphthona* spp. (Hansen, unpubl. data).

Mating occurs on leafy spurge shoots, after which adult females lay eggs at the soil surface or in the soil, on or near the base of a leafy spurge stem. Generally, *Aphthona* spp. females lay a total of 100-300 eggs during their lifetime, in a series of small groups every 3-5 days (Maw 1981). Larvae hatch, burrow into the soil, and begin feeding on very small leafy spurge roots and root hairs. As they develop, *A. czwalinae* larvae utilize progressively larger spurge roots; mature larvae may also be found burrowing within large lateral roots and root buds. Larval root feeding continues through the summer and into the fall, until cold temperatures and the onset of dormancy. There are a total of three larval stadia.

4.7 Host range in the field

Aphthona czwalinae appears to feed only on leafy spurge (*Euphorbia esula*) and several other closely-related *Euphorbia* spp. in its native Europe (Gassman 1984). To

date, introduced US populations of *A. czwalinae* and *A. lacertosa* have been reported only from *E. esula*.

4.8 Host range in laboratory/greenhouse tests

Laboratory and controlled field studies showed at least limited feeding by *Aphthona czwalinae* adults on the foliage of a number of European and North American *Euphorbia* spp. (Gassman 1984). However, only a few European *Euphorbia* spp. in the subgenus *Esula* supported larval development and thus could be considered likely hosts (Gassman 1984). No North American *Euphorbia* spp. in the subgenus *Esula* were included in these host specificity tests. Poinsettia (*E. pulcherrima*), an important horticultural plant, was not utilized by *A. czwalinae*.

Thus, the host plant range of *Aphthona czwalinae* appears restricted to the subgeneric level, and may only include leafy spurge and other Eurasian *Euphorbia* species in the subgenus *Esula*.

4.9 Specific references on the organism

Gassman, A. 1984. *Aphthona czwalinae* Weise (Coleoptera: Chrysomelidae): a candidate for the biological control of leafy spurge in North America. Intl. Inst. of Biol. Contr., Delémont, Switzerland. Final screening report. 20 p.

4.10 List of known parasitoids or predators of organism

Consumption of *Aphthona* spp. larvae and adults by generalist predators, particularly ants, has been reported anecdotally. No native or introduced parasitoids have been reported among *A. czwalinae* populations in the US.

5. Distribution of organism in the US

5.1 Current North American distribution

Aphthona czwalinae was approved for US release in July 1987. Through 1994, mixed *A. czwalinae*/*A. lacertosa* populations have been released in 15 US states: Colorado, Idaho, Iowa, Minnesota, Montana, Nebraska, New Mexico, Nevada, North Dakota, Oregon, South Dakota, Utah, Washington, Wisconsin, and Wyoming. Established populations are present in all states except ID, NV, NM, SD, and UT. In several states, *A. czwalinae* and *A. lacertosa* have been widely distributed throughout spurge-infested counties. These species also have a limited distribution in central and western Canada.

5.2 Expected North American range

There are no obvious climatic or ecological barriers to survival and establishment of *Aphthona czwalinae* in most or all of the spurge-infested areas of the US and Canada. However, *A. czwalinae* appears adapted to sites that are more mesic than those best suited to other introduced *Aphthona* spp. The largest *A. czwalinae* populations should be expected where precipitation patterns and/or soil conditions favor comparatively higher moisture levels. Of course, the ultimate North American range of this insect will reflect the extent of human redistribution activities.

6. Expected environmental impact of proposed release(s)

6.1 Human impacts

Aphthona czwalinae releases should have no impact on humans or on private property, exclusive of impacts on leafy spurge infestations.

6.2 Direct impacts

Under optimal site conditions, *Aphthona czwalinae*/*A. lacertosa* populations will, directly or indirectly, kill leafy spurge plants over large areas. Concurrently, the relative abundance of nontarget grasses and forbs will increase. Leafy spurge control over at least several acres has been reported from some locations in the western US where *A. czwalinae* and *A. lacertosa* were released.

The host range of *Aphthona czwalinae* is limited to a subset of plant species in the subgenus *Esula* of the genus *Euphorbia*, including the target weed (leafy spurge) and cypress spurge (*E. cyparissias*), an introduced weed in eastern North America. The two federally-protected native spurges (*Euphorbia garberi* and *E. deltiodes*) are in the subgenus *Chamaesyce* (Pemberton 1985) and are not potential host plants for *A. czwalinae*.

The potential host status of 21 North American species (occurring north of Mexico) in the subgenus *Esula* has not been evaluated, including *E. purpurea* and *E. telephiodes*, two rare species being considered for protection (Pemberton 1985). Of these, nine are annuals that could possibly be utilized by *A. czwalinae*, but would not permit completion of the life cycle and, hence, population establishment; flea beetle larvae require plant roots year-round. The 12 perennial species in the subgenus could be considered

potential *A. czwalinae* hosts, though most occur in the southern US and are not sympatric with leafy spurge populations (Pemberton 1985).

6.3 Indirect effects

No native or exotic insects, birds, reptiles, or mammals are known to depend largely or exclusively on leafy spurge. Thus, *Aphthona czwalinae*/*A. lacertosa* releases should have no adverse indirect impacts.

6.4 Methods to prevent undesired environmental effects

No undesired environmental effects are anticipated.

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