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Field Release of *Aulacidea acroptilonica* (Hymenoptera: Cynipidae), an Insect for Biological Control of Russian Knapweed (*Acroptilon repens*), in the Continental United States

Environmental Assessment, June 2008

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I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Permit Unit is proposing to issue permits for release of a gall wasp, *Aulacidea acroptilonica* V. Bel. (Hymenoptera: Cynipidae). The agent would be used by the applicant for the biological control of Russian knapweed, *Acroptilon* (*Centaurea*) *repens* (L.) DC, in the continental United States. Before permits are issued for release of *A. acroptilonica*, APHIS must analyze the potential impacts of the release of this agent into the continental United States.

This environmental assessment[1] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *A. acroptilonica* to control infestations of Russian knapweed within the continental United States. This EA considers the potential effects of the proposed action and its alternatives, including no action.

The applicant's purpose for releasing *A. acroptilonica* is to reduce the severity of infestations of Russian knapweed in the United States. Russian knapweed, *Acroptilon repens*, is native to Eurasia and is common in Armenia, Turkestan, Mongolia, Asia Minor, and Iran (Watson, 1980). The weed was first introduced into North America in 1898 and by 1998 had spread to 313 counties in 45 of the 48 contiguous states in the United States (Zimmerman and Kazmer, 1999). It did not become a serious weed in Canada until 1928, and its spread is linked to the distribution of knapweed-infested hay (Maddox et al., 1985). The introduction of Russian knapweed into the United States is thought to be the result of impure Turkestan alfalfa seed, and possibly sugarbeet seed (Maddox et al., 1985).

Estimated Russian knapweed acreage for the western United States and Canada in the year 2000 totaled over 1,561 million acres with 80 percent of the acreage located in the states of Washington, Idaho, Colorado, and Wyoming (Zouhar, 2001).

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[1] Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 *et seq.*) provide that an environmental assessment "[shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

Russian knapweed is a long lived perennial in the plant family Asteraceae or sunflower family. The weed thrives in a variety of habitats and is found in both irrigated and arid environments, and in cropland, pastures, rangeland, and wasteland. Russian knapweed is a strong competitor and produces allelopathic[2] compounds that exclude other plant species, and as a result, dense (100-300 plants/square meter) infestations may develop (Ivanova, 1966). This species reproduces primarily vegetatively from a primary vertical root having numerous horizontal lateral roots with deep, vertical extensions. Reproduction by seed is apparently not extensive, although a single plant may produce over 1,200 seeds that may remain viable for 2-3 years. Seeds may aid in the long range spread of the weed through infested hay and other means.

Russian knapweed has many negative qualities. It is generally not utilized for forage because of its bitter taste, and may cause neurological disorders in horses if consumed (Young et al., 1970). It reduces wildlife habitat, suppresses other plants, and has no known beneficial qualities.

Existing Russian knapweed management options are ineffective, expensive, temporary, and have nontarget impacts. For these reasons, there is a need to identify an effective, host specific biological control organism and release it into the environment for the control of Russian knapweed.

II. Alternatives

This section will explain the two alternatives available to the APHIS, Permit Unit; no action and to issue permits for environmental release of *A. acroptilonica*. Although the Permit Unit's alternatives are limited to a decision on whether to issue permits for release of *A. acroptilonica*, other methods available for control of Russian knapweed are also described. These control methods are not decisions to be made by the Permit Unit and are likely to continue whether or not permits are issued for environmental release of *A. acroptilonica*. These are methods presently being used to control Russian knapweed by public and private concerns.

A third alternative was considered, but will not be analyzed further. Under this third alternative, the APHIS Permit Unit would have issued permits for the field release of *A. acroptilonica* but the permits would contain special provisions or requirements concerning release procedures or mitigating measures. No issues have been raised that would indicate that special provisions or requirements are necessary.

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[2] Allelopathy is the inhibition of growth of one plant species by another due to the release of chemical substances.

A. No action

Under the no action alternative, the APHIS Permit Unit would not issue permits for the field release of *A. acroptilonica* for the control of Russian knapweed. The release of this biological control agent would not take place. The following methods are presently being used to control Russian knapweed and these methods will continue under the "No Action" alternative and will likely continue even if permits are issued for release of *A. acroptilonica*.

1. Chemical control

Russian knapweed may be controlled using the herbicides 2,4-D (although Russian knapweed has some tolerance of 2,4-D), picloram, dicamba, clopyralid, chlorsulfuron, metsulfuron, and clopyralid plus 2,4-D. In farmland, application of simazine at 10 kilograms/hectare in a layer 28-30 centimeters (cm) deep followed by cropping with corn for three successive years resulted in almost complete control of Russian knapweed (Tarshish and Mordovets, 1974).

2. Cultural control

Cultural controls include mowing and deep plowing. Systematic cutting of the roots to a depth of 30 cm over a three year period may destroy the root system in the top meter of soil (Mordovets et al., 1972) and root fragments up to 40 cm long may be killed by burial below 30 cm (Agadzhanyan and Agadzhanyan, 1967). Sowing desirable plant species such as smooth brome, streambank wheatgrass, thickspike wheatgrass, crested wheatgrass, or Russian wild rye is necessary after the weed is controlled with herbicides (Beck, 2007). Planting sod-forming perennial grasses, like streambank or thickspike wheatgrasses, help prevent reinvasion of Russian knapweed (Beck, 2007).

3. Biological control

The only biological control agent released on Russian knapweed in North America is the nematode *Mesoanguina picridis*, which was introduced from central Asia. The nematodes attack the shoots as they grow up through the soil and cause galls[3] to form on the stems and leaves. The galls look like tiny tennis balls, causing stunting and some mortality of Russian knapweed plants.

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[3] A gall is an abnormal growth of plant tissues caused by the stimulus of an animal or another plant.

Research and surveys by Ivanova (1966), Tyurebaev (1972), Kovalev et al. (1975), Ivannikov et al. (1976), Ivannikov and Tyurebaev (1977), Rosenthal et al. (1994), Krivokhatsky and Ovtshinnikova (1995), Sobhian (1994, 1996a,b,c), Fornasari (1996), and Schaffner et al. (2000) have identified at least 14 potential biological control organisms attacking the plant. The insects closely associated with Russian knapweed are as follows: a flower gall mite (*Aceria acroptiloni*), a vagrant mite (*Aceria sobhiani*), three flower/bud-gall flies (*Dasineura sp, Urophora xanthippe, and U. kasachstanica*), a leaf-gall weevil and midge (*Pseudorchestes* (*Rhynchaenus*) *distans* and *Loewiola acroptilonica*), a stem galling cynipid wasp (*Aulacidea acroptilonica*), stem boring beetles and a moth (*Agapanthia leucaspis, Phytoecia virgula,* and *Depressaria squamosa*), a defoliating beetle (*Galeruca interrupta armenica*), and a leaf and stem rust (*Puccinia picridis*). Root feeders associated with Russian knapweed are not well known. Three species have been reported to infest roots, but only *Cochylimorpha* (*Stenodes*) *nomadana* and *Napomyza* sp. near *lateralis* have potential as biological control agents.

B. Issue permits for environmental release of *A. acroptilonica*

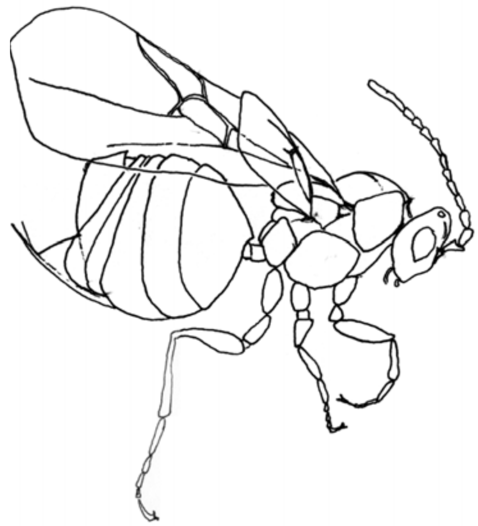
Under this alternative, the APHIS Permit Unit would issue permits for the field release of *A. acroptilonica* for the control of Russian knapweed. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

1. Biological control agent information

A. acroptilonica is a small (1.7-2.3 millimeter (mm)) gall-forming wasp belonging in the insect family Cynipidae (Figure 1). Generally gall formers are very host specific. In the literature, *A. acroptilonica* has only been reported on Russian knapweed (Kovalev and D’yakonchuk, 1986). Gall induction diverts nutrients from flower formation, seed production, and/or normal growth of plant tissues. Under certain conditions, galls may stress the plant, reducing the plant’s competitive ability and seed production, reducing long-distance spread of the weed.

A. acroptilonica has been recorded from Turkey, Georgia, southern Russia, Iran, Kazakhstan, and Uzbekistan. *A. acroptilonica* can be found in a wide range of habitats, such as along roadsides and crop fields, in orchards, vineyards, and wastelands as well as in undisturbed semi-deserts of Central Asia. Surveys in Turkey and Uzbekistan indicate that *A. acroptilonica* is most common in habitats that experience limited disturbance by animal grazing or soil cultivation. In habitats where old shoots are removed or ploughed under the soil, the gall wasp is absent or occurs in low densities.

Figure 1. Female *Aulacidea acroptilonica*



A. acroptilonica has three larval instars (immature stages). Fully developed third instar larvae are first found in late-July. The third instar larvae aestivate (a state of dormancy, similar to hibernation) over the summer, overwinter, and then pupate inside the galls in early spring. A small number of larvae remain in hibernation after the first winter, pupating after the second winter.

A. acroptilonica has one generation per year. The first adults emerge in early-April in Uzbekistan and during the second half of April in Turkey. In 2003, galls collected in Uzbekistan were kept under ambient outdoor temperature conditions at CABI Biosciences, Switzerland and then brought into the laboratory. Wasps from these galls emerged between mid-April and late-May. The sex ratio of wasps emerging from field-collected galls was strongly female biased with females making up 90-100 percent of the adults. The mean longevity of females kept at ambient room temperature and fed with sugar water was 4.9 ± 1.7 days. Newly emerged females had a mean of 160 ± 45 fully developed eggs in their abdomens. Mean egg size was 0.21 x 0.09 mm.

Based on observations in the laboratory, mating and oviposition (egg-laying) occur within hours of adult emergence. Nevertheless, unmated females successfully lay eggs, probably male. The female biased sex-ratio suggests that *A. acroptilonica*, like other Hymenoptera, are haplo-diploid, producing male adults from unfertilized eggs and females from fertilized eggs.

Eggs are laid into the meristematic tissue (tissue that is capable of cell division, resulting in growth) of the main and lateral shoots. Alighting on a plant, females walk around touching the surface of the leaves and shoots with their antennae. Once the female has reached a potential site for oviposition, she probes the plant with her ovipositor repeatedly for up to three minutes. Oviposition lasts between 10 and 70 minutes, during which the female sits motionless on the shoot tip with her head downwards and ovipositor inserted into meristematic tissue.

III. Affected Environment

Russian knapweed is a long-lived perennial. This species reproduces primarily vegetatively from a primary vertical root having numerous horizontal lateral roots with vertical extensions. The extensive root system that gives rise to new shoots allows for rapid colonization and survival value. Root depth may reach 5-7 meters, although this is uncommon. Shoots emerge early in the spring shortly after soil temperatures remain above freezing. After emergence, the plants form rosettes and bolt[4] in late May to mid-June. Flowering occurs from early July and will continue through the first hard freeze of the fall, given adequate moisture (Watson, 1980; Littlefield, unpub. data).

Russian knapweed does not appear to reproduce extensively by seed within a clone, but a single plant may produce over 1,200 seeds. Although it has been reported that seeds may remain viable for 2-3 years (Ivanova, 1966), it is speculated that the thick seed coat may allow the seed to remain dormant for a number of years. In addition, seeds may be spread through the feces of cattle that have ingested mature flower heads. Seeds of Russian knapweed germinate over a wide temperature range of 0.5^{o}-35^{o} C, with optimum germination occurring from 20^{o} to 30^{o} C (Brown and Porter, 1942; Ivanova, 1966; Muminov, 1967). Lateral spread of Russian knapweed clones is somewhat limited, approximately 35 cm per year for clones observed in Montana (Littlefield, unpubl. data) and up to 1 meter in Wyoming.

A. Areas affected by Russian knapweed

1. Native range

Russian knapweed is native to central Asia. The weed extends more or less in a band between 40^{o} and 45^{o} longitude from central Turkey and Crimea into western Mongolia and Siberia in the east, and is common in Armenia, Turkmenistan, Uzbekistan, Kazakhstan, and parts of Turkey and Iran. Russian knapweed has spread within and to adjacent areas via trade routes.

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[4] The rapid growth of a stem prior to flowering.

2. Present distribution in North America

Based on a 1998 survey (Zimmerman and Kazmer, 1999), Russian knapweed currently infests 313 counties in 45 of the 48 contiguous states in the United States. The most severe infestations of Russian knapweed occur in the more arid areas of the western United States. Estimated Russian knapweed acreage for the western United States and Canada in the year 2000 totaled over 1,561 million acres, with 80 percent of the acreage located in the states of Washington, Idaho, Colorado, and Wyoming (Zouhar, 2001). The northern distribution of Russian knapweed appears to be southern Canada, approximately 54^{o} N latitude.

3. Potential distribution

Based on a 1982 and a 1998 survey, there has been a 28.3 percent increase in number of infested counties in just 16 years (Maddox et al., 1985; Zimmerman and Kazmer, 1999)

In addition, the severity of infestations also appears to be increasing (Zimmerman and Kazmer, 1999). The potential distribution in North America is not known, but it appears that the more arid regions of the West are more susceptible to infestations.

The mode of spread has not been investigated, although long range spread of the weed is thought to occur by the transport of seeds in infested hay or crop seeds (Rogers, 1928; Renney, 1959). In addition, the ingestion of flower heads by cattle and the ability of the seeds to survive through the digestive system may also serve to disperse this weed with the movement of cattle.

4. Habitat

Russian knapweed thrives in a variety of habitats and is found in both irrigated and arid environments, and in cropland, pastures, rangeland, shrublands, and wasteland (Rogers, 1928; Zouhar, 2001). Habitat associations for the western United States may be found in Zouhar (2001). Rogers (1928) suggested that a damp clay soil would provide abundant moisture and would permit easy penetration of roots of Russian knapweed. However, Russian knapweed is found in a variety of soil types and is not associated with a particular soil. Russian knapweed is an adaptable plant and is capable of establishing itself in sandy deserts, compacted soils, and roadsides. Resistant to drought, salt, and compacted soils (Ivannikov et al, 1976), Russian knapweed can become established in areas of disturbed land or where the upper layer of soil is removed.

Examples of some perennial grass species that are commonly driven out by Russian knapweed include rough fescue (*Festuca scabrella*), Idaho fescue (*Festuca idahoensis*), bluebunch wheatgrass (*Agropyron spicatum*), western needlegrass (*Stipa occidentalis*), and Richardson's needlegrass (*Stipa richardsonii*) (Rice et al., 1992). An example of a riparian community in which Russian knapweed can commonly be found is the Freemont cottonwood (*Populus freemontii*)/skunkbrush (*Rhus trilobata*) community (TNC, 2000).

B. Plants related to Russian knapweed and their distribution

1. Taxonomically related plants

The tribe Cardueae (Russian knapweed belongs to this tribe) is comprised of approximately 83 genera and 2,500 species (Bremer, 1994). This tribe is the more primitive of Asteraceae tribes. Species are primarily Palearctic (European, the northwest coast of Africa, and Asia north of the Himalaya Mountains) and north African, although a few species are found in North and South America, Australia, and tropical Africa. The tribe Cardueae is comprised of two large subtribes - the Centaureinae and Carduinae, and two smaller subtribes – the Carlininae and Echinopsidinae. Although there are native North American species contained within the Cardueae, this tribe is comprised primarily of exotic species of economic importance either as weeds or as ornamentals and commercial crops.

In the subtribe Centaureinae, the genus *Acroptilon* consists of a single species, *Acroptilon repens* (Russian knapweed). The closely related genus *Centaurea* is comprised of approximately 32 species in the United States, mostly exotic species that are weedy or some that are used as ornamentals. There are two native knapweed species, *C. americana* and *C. rothrockii,* (some botanists have classified these under a different genus - *Plectocephalus*) of concern. Both species are annuals and are native to the southwest, although the range of *C. americana* extends up through the central United States. Both species are commercially available and may be grown as ornamentals. Safflower, *Carthamus tinctorius,* is also placed in this subtribe and is of concern due to its economic importance. The other subtribe Carduinae contains native species within the genera *Cirsium* and *Saussurea*. Six federally listed threatened or endangered *Cirsium* species are present in the United States. Artichoke, *Cynara scolymus,* is also placed in this subtribe and is of economic concern. Milk thistle, *Silybum marianum,* although considered a noxious weed in many areas is also used as an economic plant species in some locations. The subtribes Carlininae and Echinopsidinae have few representative species in North America, of which all are introduced weeds or ornamentals (i.e., globe thistle).

Besides *Cirsium*, a number of genera within the family Asteraceae have federally threatened and endangered species in the continental United States. These genera are: *Ambrosia, Artemisia, Baccharis, Blennosperma, Boltonia, Brickellia, Chromolaena, Chrysopsis, Deinandra, Echinacea, Enceliopsis, Erigeron, Eriophyllum, Grindelia, Hazardia, Helenium, Helianthus, Holocarpha, Hymenoxys, Lasthenia, Layia, Lessingia, Liatris, Malacothrix, Marshallia, Monolopia, Pentachaeta, Pityopsis, Pseudobahia, Senecio, Solidago, Stephanomeria, Symphotrichum, Taraxacum, Thymophylla, Townsendia, Verbesina,* and *Yermo*. These genera are more distantly related to *Acroptilon* and therefore would be at lower risk of being utilized by Russian knapweed biological control agents.

IV. Environmental Consequences

A. No action

1. Impact of spread of Russian knapweed

a. Beneficial uses:

Russian knapweed has no known beneficial qualities. At one time, it was recommended for soil erosion control in Nevada, that allowed the weed to become more widespread (University of Nevada Cooperative Extension, 2004).

b. Social and recreational use:

Russian knapweed does not have any known social or recreational use. The monocultural stands of this weed are considered aesthetically unpleasant in comparison to healthy ecosystems. Russian knapweed can form relatively dense stands along river beds which can inhibit recreational activities.

c. Economic losses:

Economic losses due to Russian knapweed infestations have not been studied in detail. Hirsch and Leitch (1996) addressed direct and secondary economic impacts associated with several knapweed species in Montana. Although this study was limited by various assumptions, it does indicate that potential and severe economic impacts could result should these weeds continue to spread.

Russian knapweed imparts a bitter taste to bread when its seeds are threshed together with wheat (Ivannikov et al., 1976). The quality of flour or other grain products that have been contaminated by Russian knapweed seed at a rate of only 0.01 percent by weight is reduced due to the bitter taste. At a density of 25-50 Russian knapweed plants per square meter, wheat yields were decreased by 50-90 percent (Streibig et al., 1989). Shoot densities of 19, 32, and 65 per square meter have reduced the fresh weight yield of corn by 64, 73, and 88 percent, respectively (Berezovskii and Raskin, 1971). Russian knapweed is aggressive and difficult to control in alfalfa, clover, other forage crops, and pastures. It is generally avoided by grazing animals as it imparts a bitter quinine-like taste. The presence of Russian knapweed in hay decreases the feeding value and market value.

d. Health issues:

Russian knapweed has been known to cause the neurological disorder nigropallidal encephalomalacia in horses (Young et al., 1970). No known human ailments have been associated with Russian knapweed, although the plant produces repin and acroptilin, allergenic sesquiterpene lactones which may cause dermal allergies with prolonged or repeated contact.

e. Effects on wildlife populations:

Kurz et al. (1996) reported on the ecological implications of Russian knapweed infestations on small mammals and habitat associations. Field sites in Wyoming and Colorado were chosen for the study of diversity comparisons at infested and non-infested sites. Vegetation measurements indicated distinct differences in composition and structure between knapweed infested and non-infested plots. Diversity comparisons showed a large shift in species composition in Russian knapweed infested areas for both small mammal and plant communities, indicating a displacement of native species.

Certain species of wildlife may, in certain cases, utilize Russian knapweed infested habitats more. A recent study in Colorado and Wyoming indicates that three times as many small mammals frequented Russian knapweed infested rangeland compared to adjacent non-infested sites. Adaptation to Russian knapweed infested sites sometimes occurs, as evidenced by one small mammal - a harvester mouse, which utilizes seeds, and may serve to spread the weed as they cache seeds. In contrast, Russian knapweed infested areas had severely reduced populations of kangaroo rats and ground squirrels in Wyoming (Johnson et al., 1994).

Hirsch and Leitch (1996) estimate that knapweed monoculture could reduce wildlife habitat values as much as 80 percent. Russian knapweed infestations have also been reported to impact big horn sheep forage in British Columbia (Zouhar, 2001).

2. Impact from use of other control methods

a. Chemical control

The continued use of chemical herbicides and mechanical controls at current levels would be a result if the “no action” alternative is chosen.

Although herbicide treatments are temporarily effective, they are short term solutions that must be repeated (Jones and Evans, 1973; Gruzdev and Popov, 1974; Krumzdorov, 1976; Alley and Humberg, 1979, Benz et al., 1996). In addition, a one-time application of herbicide is usually insufficient in managing Russian knapweed.

Large scale chemical control is potentially ecologically harmful and often not economical on western rangeland, which is of relatively low productive value (DiTomaso, 2000). In Fremont County, Wyoming alone, very conservative estimates to apply one herbicide treatment on Russian knapweed (approximately $15/acre), would exceed $950,000 (Baker et al., 1999). This estimate is considered conservative because it does not factor in the additional cost of treating remote infestations. The majority of infested acres in Fremont County are considered remote grazing land (Baker et al., 1999).

b. Mechanical control

The control of this perennial weed by deep plowing or by mowing may have a limited affect on its extensive root system while disturbing or destroying nontarget plants. Mowing appears to stimulate regrowth in the aerial portion of the plant and may induce dormancy in the roots (Tarshish, 1967). Russian knapweed clones are able to compensate for artificial destruction of individual members of the clone by regrowth from root buds (Schaffner et al., 2001). Cultural control of Russian knapweed is typically not economical on low-productive value rangeland.

c. Biological control

The only biological control agent released on Russian knapweed in North America has been the nematode species *Mesoanguina* (*Subanguina*) *picridis*, which was introduced from central Asia and released in the United States in 1984. Laboratory experiments suggested extensive damage on the seed development and plant growth from this agent. Due to the low mobility of the nematode and varying moisture conditions, the results of field releases were less than expected. *Mesoanguina picridis* would need to be propagated and redistributed on a large scale, which would not be cost effective. For these reasons other organisms are being considered for biological control.

These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *A. acroptilonica* to reduce Russian knapweed in the continental United States. It is not expected that *A. acroptilonica* alone will completely control Russian knapweed. However, reductions in the above-ground growth rate and seed output caused by *A. acroptilonica* are expected to lead to reduced competitive ability of the weed and reduced long-distance dispersal of Russian knapweed seeds.

B. Issue permits for environmental release of *A. acroptilonica*

1. Impact of *A. acroptilonica* on nontarget plants

Host specificity to Russian knapweed has been demonstrated through scientific literature, field observations, and host specificity testing.

a. Scientific literature

According to Kovalev and D’yakonchuk (1986), *A. acroptilonica* only attacks Russian knapweed.

b. Field observations

During field surveys in Turkey (Collier et al. 2006), several hundred individual *Centaurea cyanus*, *Carduus pycnocephalus,* and *Cirsium* species (plant species that are closely related to Russian knapweed) were inspected for galls in habitats where *A. acroptilonica* occurs naturally. None of the inspected plant species were attacked by this gall wasp. These data suggest that *A. acroptilonica* is likely to attack only Russian knapweed in its native range.

c. Host specificity testing

<u>Site of quarantine and field studies</u>

All laboratory tests were conducted at the CABI Bioscience Centre in Delémont, Switzerland. The open-field experiments were carried out in Uzbekistan and Turkey.

<u>Test plant list</u>

The test plant list used to determine the host specificity of *A. acroptilonica* consisted of the target population of Russian knapweed collected from various localities in Wyoming and 48 nontarget test plant species or varieties. The test plant list was constructed with the aim of including at least one representative of the major tribes of the family Asteraceae and one representative of genera within the same tribe as Russian knapweed. The test plant list included the two native North American species in the genus *Centaurea* (*C. americana* and *C. rothrockii*) and Eurasian species of the genus *Centaurea* that are used as ornamentals in North America (*C. cyanus*, *C. montana*). By all accounts, members of the genus *Centaurea* are considered to be the closest relatives of *Acroptilon* in North America.

In developing the test list, emphasis was also placed on including as many native North American representatives of the various taxa as possible. Of the three subfamilies of the Asteraceae, only the Cichorioideae and the Asteroideaea contain indigenous species in North America (Bremer, 1994). Because Russian knapweed resides within the Cichorioideae, this subfamily was more extensively tested than the Asteroideae. Additionally, a greater number of genera and species within the tribe Cardueae and subtribe Carduinae were tested than those in other tribes and subtribes. The assumption here is that the former taxa are more closely related to *Acroptilon* and so would be more likely to be potential host plants for *A. acroptilonica*. Furthermore, the Cardueae and Carduinae contain a number of plants that are native to North America, are federally-listed threatened or endangered species, or are important crop plants (e.g. artichoke, lettuce, safflower).

<u>Laboratory tests</u>

In the laboratory, host specificity tests followed a sequential no-choice design. Potted nontarget test plants and Russian knapweed plants were covered with plastic cylinders (10 cm diameter; 15-100 cm high), each topped with a gauze lid. Two females each were introduced into the plastic cylinders for two days. Female wasps were removed and then transferred onto the next test or control plant. Dead females were replaced with newly emerged females. Care was taken that a pair of gall wasps was never exposed to the same test plant species more than once. After exposure, test and control plants were put back into the garden, where they were regularly inspected for gall formation.

Table 1 shows the results from the sequential no-choice laboratory tests with the *A. acroptilonica* populations from Turkey and Uzbekistan. No galls were produced on any of the nontarget test plants (Table 1) with the exception of a single gall on *Centaurea americana*. Galls were readily induced on the target plant Russian knapweed.

Table 1. Results of the no-choice oviposition/gall formation tests (1998-2004) (Collier et al., 2006).

Plant Species	Turkey Reps	Turkey Galls	Uzbekistan Reps	Uzbekistan Galls
SUBFAMILY CICHORIOIDEAE				
Tribe Cardueae				
Subtribe Centaureinae				
Acroptilon repens	**122**	**42**	**154**	**99**
Centaurea americana	**67**	**1**	**24**	**0**
C. rothrockii	23	0	9	0
C. jacea	10	0	7	0
C. solstitialis	5	0	7	0
C. cyanus	14	0	10	0
C. arenaria	5	0	6	0
C. napifolia	7	0	1	0
C. montana	12	0	9	0
Carduncellus mitissimus	13	0	8	0
Carthamus tinctorius				
Cal-West 4440	-	-	9	0
Cal-West 1221	16	0	6	0
Cal-West 88-OL	19	0	5	0
SeedTec 518	8	0	7	0
SeedTec 555	5	0	5	0
SeedTec 317	7	0	3	0
Montola 2000	15	0	5	0
Montola 2001	11	0	4	0
Serratula tinctoria	13	0	8	0
Subtribe Carduinae				
Carduus pycnocephalus	3	0	5	0
Cirsium muticum	3	0	-	-
C. hillii	2	0	-	-
C. vinaceum	5	0	-	-
C. fontinale	2	0	-	-
C. arvense	13	0	14	0
C. pannonicum	6	0	-	-
C. undulatum	7	0	-	-
C. vulgare	3	0	3	0
C. discolor	18	0	7	0

(**Table 1**, continued)

Plant Species	Turkey		Uzbekistan	
	Reps	Galls	Reps	Galls
Subtribe Carduinae (cont.)				
C. canescens	-	-	1	0
C. hydrophilum	1	0	2	0
Cynara scolymus				
Green Globe	10	0	3	0
Viletto			2	0
Silybum marianum	12	0	9	0
Subtribe Carlininae				
Carlina vulgaris	-	-	9	0
Subtribe Echinopsidinae				
Echinops rito	13	0	10	0
Tribe Lactuceae				
Subtribe Crepidinae				
Taraxacum officinale	10	0	9	0
T. laevigatum	9	0	-	-
Subtribe Hieraciinae				
Hieracium canadense	8	0	10	0
Subtribe Lactucinae				
Cichorium intybus	8	0	9	0
Lactuca sativa	23	0	8	0
Subtribe Sonchinae				
Sonchus arvensis	4	0	1	0
Subtribe Stephanomeriinae				
Stephanomeria virgata	10	0	-	-
Tribe Vernonieae				
Subtribe Vernoniinae				
Vernonia missourica	13	0	8	0
V. fasciculata			5	0

(**Table 1**, continued)

Plant Species	Turkey Reps	Turkey Galls	Uzbekistan Reps	Uzbekistan Galls
SUBFAMILY ASTEROIDEAE				
Tribe Astereae				
Aster laevis	12	0	11	0
A. umbellata	13	0	2	0
Solidago nemoralis	12	0	8	0
Erigeron pinnatisectus	10	0	3	0
Tribe Anthemideae				
Artemisia ludoviciana	10	0	15	0
Tribe Senecioneae				
Senecio fremontii	-	-	2	0
S. triangularis	7	0	-	-
S. pauperculus	12	0	-	-
Cacalia atriplicifolia	16	0	5	0
Tribe Helenieae				
Helenium autumnale	13	0	10	0
H. flexuosum	12	0	5	0
Hemizonia conjungens	3	0	-	-
H. pungens	12	0	9	0
Gaillardia pinnatifida	10	0	13	0
Tribe Helinatheae				
Helianthus annuus				
Cargill SF 187	12	0	8	0
Cargill SF 270	16	0	4	0
Echinacaea pallida	8	0	4	0
E. purpurea	12	0	4	0
Liatris pycnostachya	9	0	-	-
Tribe Eupatorieae				
Eupatorium maculatum	10	0	11	0
E. perfoliatum	15	0	10	0
Tribe Calenduleae				
Calendula officinalis	12	0	12	0
Tribe Gnaphalieae				
Gnaphalium audax	7	0	1	0
G. obtusifolium	12	0	7	0

Field tests - multiple-choice oviposition and gall formation

In addition to the laboratory no-choice tests, multiple-choice oviposition and gall formation tests were conducted under open-field conditions in Turkey and Uzbekistan. Test plant species were either grown from seed or collected and transplanted from the local area. Plants were arranged with Russian knapweed in a randomized design at one of three localities, one in Turkey and two in Uzbekistan. Galls of *A. acroptilonica* were collected locally and brought out in the field plots in a way to minimize losses due to wind dispersal and predation.

In the open-field experiments, gall formation was recorded from Russian knapweed and *Centaurea americana* but from no other test plant species (Table 2). The three galls produced on *C. americana* occurred on a single plant in a single replicate during 2000. No galls were produced on any of the nontarget test plants, including *C. americana,* in any replicates in the 2001 tests in Uzbekistan nor in the 2002 tests in Turkey. Galls produced on *C. americana* were preserved in alcohol; therefore it was not possible to determine if these gall wasps would have successfully emerged from *C. americana* as viable adults. Also it was not possible to determine if the galls were produced by *A. acroptilonica* rather than some other naturally occuring cynipid.

Table 2. Results of the multiple-choice, open-field oviposition and gall formation tests carried out in Uzbekistan (2001) and Turkey (2000 and 2002) (Collier et al., 2006).

Plant species	Replicates	Number of galls
A) Turkey (2000)		
Acroptilon repens	20	7
Centaurea americana	10	3
C. rothrockii	12	0
C. solstitialis	22	0
C. nigra	10	0
Carduus pycnocephalus	10	0
Silybum marianum	10	0
Carthamus lanatus	10	0
C. tinctorius	29	0
Echinops sp.	17	0
Cirsium acarna	13	0

Table 2 **(continued)**

Plant species	Replicates	Number of galls
B) Uzbekistan (2001)		
Acroptilon repens	9	30
Centaurea americana	9	0
Onopordum illyricum	10	0
Carthamus tinctorius	10	0
Carthamus lanatus	9	0
Cirsium vulgaris	8	0
C. arvense	10	0
C. discolor	1	0
C. acharna	9	0
C) Turkey (2002)		
Acroptilon repens	19	9
Centaurea americana	10	0
C. rothrockii	10	0
C. solstitialis	12	0
C. nigra	10	0
Carduus pycnocephalus	14	0
Cynara scolymus	10	0
Silybum marianum	10	0
Carthamus lanatus	10	0
C. tinctorius	20	0
Cirsium discolor	10	0
C. acarna	15	0
C. arvense	17	0
C. vulgare	10	0
Echinops rito	18	0
Matricaria perforata	10	0
Helianthus annuus	18	0

An impact experiment was set up with the nontarget species *Centaurea americana* at CABI Bioscience Switzerland Centre because *C. americana* is the only nontarget test plant on which gall formation was observed. A total of 58 bolting *C. americana* plants were randomly assigned to either a group exposed to the gall wasp or a group untreated as control. In parallel, nine Russian knapweed plants of similar growth stage were exposed to gall wasps. As soon as adult gall wasps started to emerge from galls stored in the laboratory, all plants were covered with plastic cylinders (10 cm diameter and 15-50 cm height). Plants from the "galled" group were exposed to two *A. acroptilonica* females for two days. After the exposure period, the wasps were aspirated, the cylinders removed and the plants transferred to the Centre's garden. None of the 29 potted *C. americana* plants exposed to the gall wasp produced galls, while gall formation was observed in six of the nine Russian knapweed plants that were exposed to the gall wasp. Hence, the potential impact of gall wasp attack on individual *C. americana* plants could not be assessed. Nevertheless, the experiment provides further evidence that attack of this nontarget species by *A. acroptilonica* is likely to be rare under no-choice conditions, as well as under sequential-choice conditions.

Discussion

The results from the laboratory and open-field tests indicate that *A. acroptilonica* has a very narrow host range. None of the nontarget test plant species except *Centaurea americana* showed any signs of gall formation. The level of attack on *C. americana* was so low that nontarget impact could not be assessed. In sequential no-choice tests, only a single gall was produced on *C. americana* test plants in 66 replicates with the Turkish population of *A. acroptilonica* and no galls were produced in 21 replicates with the Uzbek population. In the open-field choice tests, no galls were produced in 9 replicate tests in Uzbekistan and 3 galls were produced in 20 replicate tests in Turkey. All three of the galls in the Turkish tests were found on a single plant in a single replicate.

One potential explanation for the galls in the open-field tests is that they were produced by a gall wasp species other than *A. acroptilonica*. Because the three galls were preserved in alcohol, it was impossible to determine whether the galls were produced by *A. acroptilonica*. Other *Aulacidea* spp., (i.e. *A. discolor* and *A. parvula*) have been reported to attack *Centaurea* spp. in Asia but these data need to be verified.

Finally, it is unclear whether gall formation on *C. americana* leads to successful emergence of viable *A. acroptilonica* adults. Galls formed in the 2000 choice trial were preserved in alcohol. No adult gall wasps emerged from the single gall formed in the 2000 no-choice test, so data on performance of the gall wasp on *C. americana* are not available. In subsequent trials attempting to investigate this issue, it has been impossible to produce additional galls on *C. americana*.

2. Uncertainties regarding the environmental release of *A. acroptilonica*

Once a biological control agent such as *A. acroptilonica* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (Russian knapweed) to attack nontarget plants, such as native *Centaurea america*. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by *A. acroptilonica*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *A. acroptilonica* generally spread without intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur and in which the climate is suitable for reproduction and survival.

In addition, these agents may not be successful in reducing Russian knapweed populations in the continental United States. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on Russian knapweed by *A. acroptilonica* will not be known until after release occurs and post-release monitoring has been conducted. It is not expected that *A. acroptilonica* alone will control populations of Russian knapweed, but will act in combination with other control methods or biological control agents.

3. Cumulative impacts

"Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Many states and counties, particularly in the western United States, conduct weed control programs to manage Russian knapweed as well as other invasive weeds. Chemical, mechanical, and biological controls, as described previously in this document are used in a wide range of habitats.

Release of *A. acroptilonica* is not expected to have any negative cumulative impacts in the continental United States because of its host specificity to Russian knapweed. Effective biological control of Russian knapweed will have beneficial effects for weed management programs, and may result in a long-term, non-damaging method to assist in the control of Russian knapweed, and prevent its spread into other areas potentially at risk from invasion.

4. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened endangered species or result in the destruction or adverse modification of critical habitat.

Sixty-six species of Asteraceae are federally-listed as threatened or endangered in the continental United States. These represent species found within both subfamilies of Asteraceae and the majority of the Asteraceae tribes, including Cardueae.

APHIS has determined that based on the host specificity of *A. acroptilonica,* there will be no effect on any listed plant or designated critical habitat in the continental United States based on literature, field observations, and host specificity testing. In host specificity testing, the biological control agents caused gall formation only in Russian knapweed. The only exception was a few galls formed on the closely related *Centaurea americana.* No listed species occur in the genus *Centaurea.*

V. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects from the field release of *A. acroptilonica* and will not have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, "Protection of Children From Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews is involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of the field release of *A. acroptilonica*.

Executive Order (EO) 13175, "Consultation and Coordination with Indian Tribal Governments," was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications…."

In April 2008, APHIS sent out letters to tribal leaders and organizations of 6 States (Wyoming, Colorado, Montana, Idaho, Oregon, and Washington) to give notification and to request input on the proposal for environmental release of *A. acroptilonica*. A draft environmental assessment was sent with each letter. No response was received from any tribe during the comment period.

VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *A. acroptilonica* on March 5, 2007. TAG members that reviewed the release petition (Collier et al. 2006) included representatives from the U.S. Fish and Wildlife Service, Bureau of Indian Affairs, Weed Science Society of America, Cooperative State Research, Education, and Extension Service, National Park Service, U.S. Geological Survey, Environmental Protection Agency, U.S. Forest Service, U.S. Army Corps of Engineers, Bureau of Reclamation, Animal and Plant Health Inspection Service, Agriculture and Agri-Food Canada, Health Canada, and University of British Columbia.

This EA was prepared and reviewed by APHIS. The addresses of participating APHIS units, cooperators, and consultants (as applicable) follow.

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Policy and Program Development
Environmental Services
4700 River Road, Unit 149
Riverdale, MD 20737

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Permits, Registrations, Imports, and Manuals
4700 River Road, Unit 133
Riverdale, MD 20737

University of Wyoming
Department of Renewable Resources

P.O. Box 3354
Laramie, WY 82071

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TNC–see The Nature Conservancy

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Decision and Finding of No Significant Impact
for
Field Release of *Aulacidea acroptilonica* (Hymenoptera: Cynipidae), an Insect for Biological Control of Russian Knapweed (*Acroptilon repens*), in the Continental United States
June 2008

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), is proposing to issue permits for release of a gall wasp, *Aulacidea acroptilonica* V. Bel. (Hymenoptera: Cynipidae). The agent would be used by the applicant and any future permittees for the biological control of Russian knapweed, *Acroptilon* (*Centaurea*) *repens* (L.) DC, in the continental United States. APHIS has prepared an environmental assessment (EA) that analyzes the potential environmental consequences of this action. The EA is available from:

U.S. Department of Agriculture
Animal and Plant Health inspection Service
Plant Protection and Quarantine
Permits, Registrations, Imports and Manuals
4700 River Road, Unit 133
Riverdale, MD 20737
http://www.aphis.usda.gov/plant_health/ea/biocontrol_weeds.shtml

The EA analyzed the following two alternatives in response to the need to control Russian knapweed and contain infestations: (1) no action, and (2) issue permits for the release of *A. acroptilonica* for biological control of Russian knapweed. A third alternative, to issue permits with special provisions or requirements concerning release procedures or mitigating measures, was considered. However, this alternative was dismissed because no issues were raised that indicated that special provisions or requirements were necessary. The No Action alternative, as described in the EA, would likely result in the continued use at the current level of chemical, cultural, and biological control methods for the management of Russian knapweed. These control methods described are not alternatives for decisions to be made by APHIS, but are presently being used to control Russian knapweed in the United States and may continue regardless of permit issuance for field release of *A. acroptilonica.* Notice of the EA was made available in the Federal Register on April 24, 2008 for a 30-day public comment period. No comments were received on the EA.

I have decided to authorize the PPQ permit unit to issue permits for the environmental release of *A. acroptilonica.* The reasons for my decision are:

- This biological control agent is sufficiently host specific and poses little, if any, threat to the biological resources of the continental United States.
- The release will have no effect on federally listed threatened and endangered species or their habitats in the continental United States.
- *A. acroptilonica* poses no threat to the health of humans or wild or domestic animals.

- No negative cumulative impacts are expcted from release of *A. acroptilonica.*

- There are no disproportionate adverse effects to minorities, low-income populations, or children in accordance with Executive Order 12898 "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations" and Executive Order 13045, "Protection of Children from Environmental Health Risks and Safety Risks."

- While there is not total assurance that the release of *A. acroptilonica* into the environment will be reversible, there is no evidence that this organism will cause any adverse environmental effects.

An environmental impact statement (EIS) must be prepared if implementation of the proposed action may significantly affect the quality of the human environment. I have determined that there would be no significant impact to the human environment from the implementation of any of the action alternatives and, therefore, no EIS needs to be prepared.

M. J. Firko

Dr. Michael J. Firko
Director
Permits, Registrations, Imports, and Manuals
APHIS Plant Health Programs
Plant Protection and Quarantine

09 June 2008
Date