United States
Department of
Agriculture

Marketing and
Regulatory
Programs

Field Release of the Plant Fungus *Ramularia crupinae* (Deuteromycotina) for Classical Biological Control of Common Crupina, *Crupina vulgaris* (Asteraceae), in the Contiguous United States.

Environmental Assessment, December 2020

Field Release of the Plant Fungus *Ramularia crupinae* (Deuteromycotina) for Classical Biological Control of Common Crupina, *Crupina vulgaris* (Asteraceae), in the Contiguous United States.

Environmental Assessment, December 2020

Agency Contact:
Colin D. Stewart, Assistant Director
Pests, Pathogens, and Biocontrol Permits
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
4700 River Rd., Unit 133
Riverdale, MD 20737

This publication reports research involving pesticides. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish and other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended label practices for the use and disposal of pesticides and pesticide containers.

Contents

I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the leaf spotting fungus *Ramularia crupinae* Dianese, Hasan & Sobhian (Deuteromycotina). *Ramularia crupinae* would be used for the classical biological control of common crupina, (*Crupina vulgaris* Cassini) (Asteraceae), in the contiguous United States.

Classical biological control of weeds is a weed control method where natural enemies from a foreign country are used to reduce exotic weeds that have become established in the United States. Several different kinds of organisms have been used as biological control agents of weeds: insects, mites, nematodes, and plant pathogens. Efforts to study and release an organism for classical biological control of weeds consist of the following steps (TAG, 2016):

1. Foreign exploration in the weed's area of origin.
2. Host specificity studies.
3. Approval of the exotic agent by PPBP.
4. Release and establishment in areas of the United States invaded by the target weed.
5. Post-release monitoring.

This environmental assessment[1] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act of 1969 (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 *R. crupinae* to control infestations of common crupina within the contiguous United States. This EA considers the potential effects of the proposed action and its alternatives, including no action. Notice of this EA was made available in the Federal Register on October 30, 2020 for a 30-day public comment period. One comment was received on the EA by the close of the comment period. However, this comment did not raise any substantive issues.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United

[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.

States must receive PPQ Form 526 permits for such activities. The PPBP received a permit application requesting environmental release of the leaf spotting fungus, *R. crupinae,* from France, and the PPBP is proposing to issue permits for this action. Before permits are issued, the PPBP must analyze the potential impacts of the release of this agent into the contiguous United States.

The applicant's purpose for releasing *R. crupinae* is to reduce the severity of infestations of common crupina in the contiguous United States. Common crupina is spreading in pastures and rangelands resulting in a reduction in quality of forage as it displaces other species. The present infestations are small compared to those of other invasives, but the potential for large, dense populations throughout the United States is considerable and is justification for its status as a noxious weed. Originally, five infestations of common crupina were reported in the United States, all presumably originating from Spain during the 1930s or 1940s via Basque shepherds (Roché et al., 2003). Common crupina is spreading, as evidenced from the first discovery in 1968 near Grangeville, Idaho, where it was recorded on about 18 hectares (ha) of rangeland (Stickney, 1972) in Idaho County. After 22 years, the original infestation was greater than 25,000 ha (Garnatje et al., 2002; Roché et al., 2003), and currently there are eleven counties infested in Idaho alone (USDA-NRCS, 2007). Further evidence of regional spread is the discovery of two locations in Wallowa County, Oregon. Furthermore, the known distribution of *C. vulgaris* is likely to be a low estimate, because the crupina plant has a thin stature and individual plants or small infestations are very difficult to see among the mixture of rangeland and pasture plant populations (Gamarra and Roché, 2002). It is likely, therefore, that there are many small infestations (including individual plants) that have not been recognized to date (Roché et al., 2003).

Existing options for management of common crupina, such as herbicides and tillage, although effective, are often impractical because of issues concerning size, terrain, or ecological sensitivity of infestations (Prather and Callihan, 1993). For these reasons, the applicant has a need to release *R. crupinae,* a host-specific, biological control organism for the control of common crupina, into the environment.

II. Alternatives

This section will explain the two alternatives available to the PPBP—no action and issuance of permits for environmental release of *R. crupinae*. Although the PPBP's alternatives are limited to a decision on whether to issue permits for release of *R. crupinae*, other methods available for control of common crupina are also described. These control methods are not decisions to be made by the PPBP, and their use is likely to continue whether or not permits are issued for environmental release of *R. crupinae*, depending on the efficacy of *R. crupinae* to control common crupina. These are methods presently being used to control common crupina by public and private concerns.

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

A. No Action

Under the no action alternative, PPBP would not issue permits for the field release of *R. crupinae* for the control of common crupina. The release of this biological control agent would not take place. The following methods are presently being used to control common crupina; these methods will continue under the "No Action" alternative and will likely continue even if permits are issued for release of *R. crupinae*, depending on the efficacy of the organism to control common crupina.

1. Prevention

Prevention is the most important measure for management, including use of hay that is certified seed-free, and quarantining horses and cattle for six days after grazing on common crupina-infested pasture (Thill et al., 1986).

2. Chemical Control

Commonly used herbicides to control common crupina are: dicamba plus 2,4-D, glyphosate, clopyralis, triclopyr, metsulfuron, and picloram. Herbicides need to be applied repeatedly, for at least three years, in order to eliminate the seed bank.

3. Mechanical Control

Common crupina can be pulled by hand or dug when the soil is moist when infestations are small.

4. Cultural Control

Establishment of selected grasses can be an effective cultural control of common crupina (CDA, undated). Healthy plant communities can deter common crupina from establishing.

B. Issue Permits for Environmental Release of *R. crupinae*

Under this alternative, the PPBP would issue permits for the field release of the leaf spotting fungus *R. crupinae* for the control of common crupina. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Biological Control Agent Information

1. Taxonomy

Kingdom:	Fungi
Subkingdom:	Dikarya
Phylum:	Ascomcota
Subphylum:	Pezizomycotina
Class:	Dothideomycetes
Subclass:	Dothideomycetidae
Order:	Capnodiales
Genus:	*Mycosphaerella*
Genus:	*Ramularia*
Species:	*crupinae*

(Hibbett et al., 2007)

2. Description of *R. crupinae*

Ramularia crupinae was described by Dianese, Hasan and Sobhian (Dianese et al., 1996). It has only been collected in Montferrier-sur-Lez, France, and it differs from other *Ramularia* species associated with the plant family Asteraceae on the basis of size of conidia (a fungus spore produced asexually), and both shape and position of stromata (cushionlike plate of solid mycelium (masses of filaments that form the body of a typical fungus)) (Dianese et al., 1996). The isolate for this evaluation (IMI 360424) was provided by R. Sobhian, the collector and co-author on the manuscript describing this species.

3. Geographical Range of *R. crupinae*

Ramularia crupinae has been reported only from France (Dianese et al., 1996; Hasan et al., 1999).

4. Life History of *R. crupinae*

As a member of the Fungi Imperfecti (Deuteromycetes), there is no known sexual form of *Ramularia* species. The fungus overwinters in debris from infected plant parts. Under moist warm conditions in the spring and summer, substomatal stroma develop that produce conidiophores (an asexual reproductive structure that produces conidia). The conidia are liberated by wind or rain, and thus carried to healthy plants. When temperatures are conducive and there is moisture (rain or dew), the spores germinate, form appressoria (specialized cells that are used by plant pathogens to infect host plants), and attempt to invade the plant. If the host is susceptible, the infection will proceed and result in disease. From these infections, the next generation of conidia will be produced and spread to new plants.

III. Affected Environment

A. Taxonomy and Description of Common Crupina

Division:	Magnoliophyta
Class:	Magnoliopsida
Family:	Asteraceae
Subfamily:	Cichorioideae
Tribe:	Cardueae (Cynareae)
Subtribe:	Centaureinae
Genus:	*Crupina*
Species:	*vulgaris*
Varieties	*vulgaris* and *brachypappa* Beauvois

Common names: common crupina, bearded creeper
Scientific name: *Crupina vulgaris* Cassini
Synonyms: *Centaurea crupina*, *Serratula crupina*

Only *C. vulgaris* occurs in the United States (Roché et al., 2003). In the United States there are two varieties of *C. vulgaris* (USDA-NRCS, 2007). Biological and developmental characteristics of each variety are distinct and remain true from one generation to the next (Roché and Thill, 2001). The two varieties are *C. vulgaris* var. *vulgaris* and *C. vulgaris* var. *brachypappa* (Couderc-LeVaillant and Roché, 1993).

Common crupina is a winter annual that may grow from 0.3 to 1.0 meters (m) in height. Rosettes develop through the fall and winter, and plants bolt in the spring (Roché et al., 1997a). Flowers are lavender to purple, 1–2 centimeters (cm) long, and inconspicuous. Each plant produces a range of 3 to 27 capitula (a dense, flat cluster of small flowers) and 2 to 23 achenes (small, dry, one-seeded fruits) in a dry Idaho grassland environment, compared to 250 to 400 capitula and 800 to 850 achenes in more moist sites from Oregon and Washington (in Roché and Thill, 2001). One estimate for yield was about 1,000 achenes per square meter (Roché and Thill, 2001). Seeds, generally one per head, are black to silvery beige, very large (3–4 millimeters (mm) long), cylindrical, and taper slightly to a blunt end. One end of each seed has a tuft of short stiff, dark hairs (Thill et al., 1987; Keil and Turner, 1993).

Distinct morphological (structural) differences occur between the two varieties of common crupina in the United States (Roché et al., 1997a). Seed weight, length of achenes, and hairs on the seed differ between varieties. Achenes of var. *vulgaris* are heavier, longer, and have longer hairs on the seeds, measuring 4.52 ± 0.13 mm long, with the longest seed hair measuring 7.98 ± 0.19 mm, vs. 4.16 ± 0.10 and 5.14 ± 0.11 mm, respectively, for var. *brachypappa* (Couderc-LeVaillant and Roché, 1993).

Variety *vulgaris* is lighter green in color, as evidenced by lower chlorophyll content, and rosettes tend to be more upright, compared with var. *brachypappa*, which has dark, shiny green leaves in flat rosettes (Figure 1).

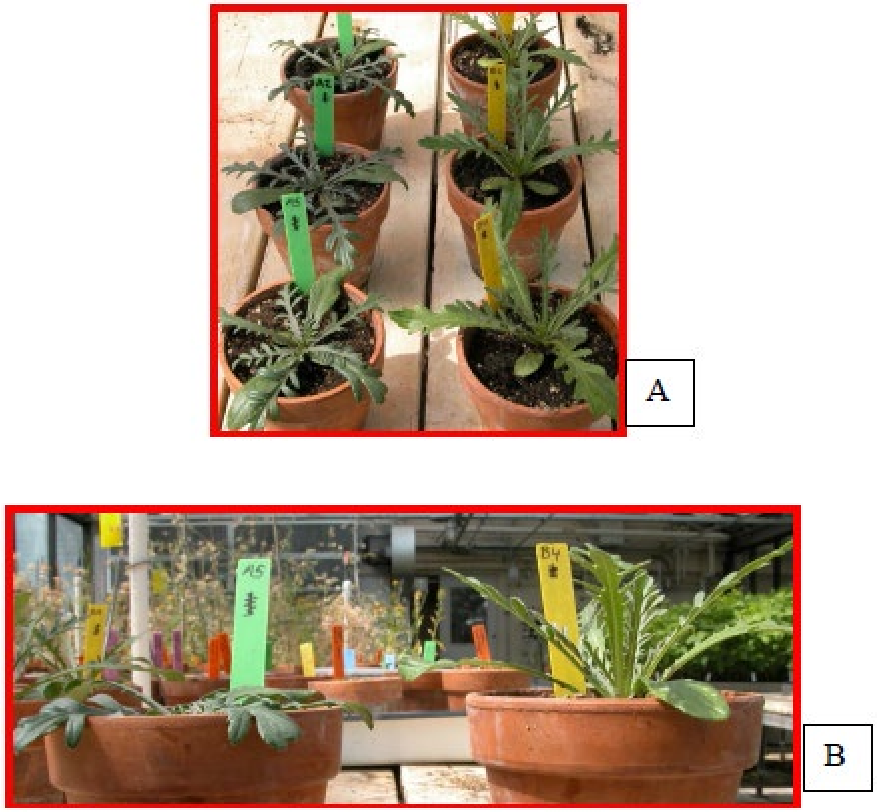

Figure 1. Growth characteristics of *Crupina vulgaris* varieties. **A.** Flat rosettes and dark green color of *C. vulgaris* var. *brachypappa* from Modoc Co., CA (left) compared to "upright" rosettes and lighter green color of *C. vulgaris* var. *vulgaris* from Salmon River, ID. **B.** Side views of both varieties showing the flat rosette of *C. vulgaris* var. *brachypappa* (left) and the "upright" rosette of *C. vulgaris* var. *vulgaris*. (Bruckart, 2013)

B. Areas Affected by Common Crupina

1. Native Range of Common Crupina

Common crupina is a native of Eurasia, most likely originating in the Middle East. The native distribution includes the Mediterranean region as far west as the Iberian Peninsula and east into Uzbekistan (Roché et al., 2003).

2. Introduced Range of Common Crupina

Common crupina has been introduced into both the United States and Australia (in: Roché, et al., 2003), and it is an economically important pest in the rangelands of southern Russia (Thill et al., 1987). In the United States, it presently occurs only in California, Idaho, Oregon, and Washington (Ganatje et al., 2002; Roché et al., 2003; USDA-NRCS, 2007) (Figure 2). Although populations remain discrete in the United States, the plant is persistent and continues to spread at each location (Garnatje et al., 2002; Roché et al., 2003).

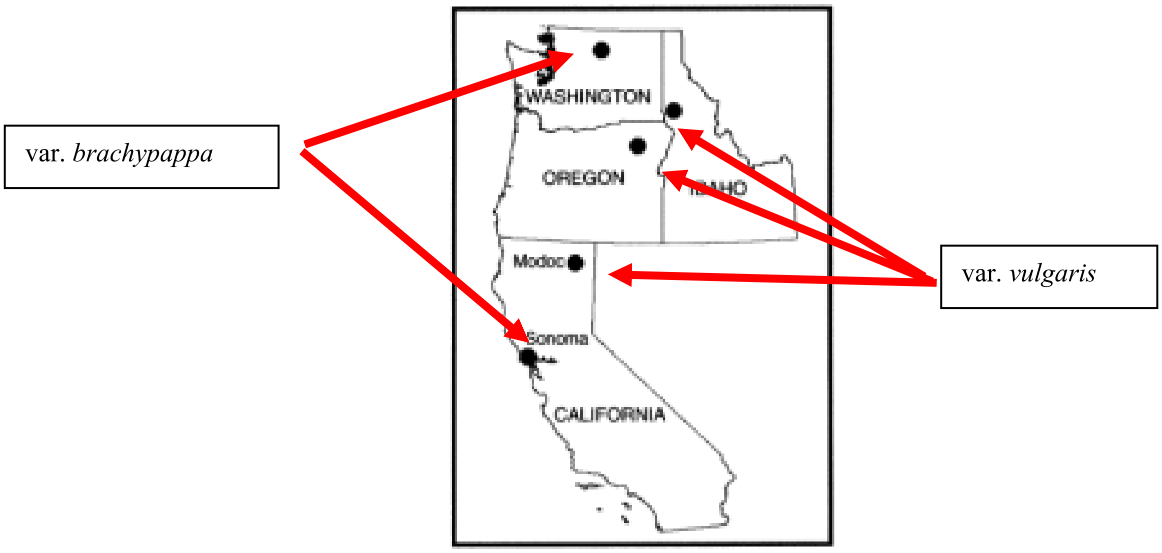

Figure 2. Distribution of *Crupina vulgaris* var. *vulgaris* and *C. vulgaris* var. *brachypappa* in the United States (From: Garnatje et al., 2002).

3. Life History of Common Crupina

Common crupina is a winter annual. Seeds germinate in the fall and the plant forms rosettes. Plants winter over as rosettes, and they bolt and flower from mid-May to early June (DiTomaso and Healy, 2007; Roché et al., 1997a).

Even though common crupina is a preferential outcrosser and generally relies on insects for cross-pollination, it is also facultatively autogamous (self-fertilizes). Stigmas become receptive to selfing shortly before the corolla withers (Roché and Thill, 2001; DiTomaso and Healy, 2007), and for this reason, seed production occurs even without insect pollination. Achenes mature in June (Roché et al., 1997a). Common crupina reproduces entirely by seed. Although common crupina only produces a few seeds compared to other species, it is successful because the seeds are large, of high quality, have high germination rates in nature (Roché and Thill, 2001; Roché et al., 2003), and there is very little seedling mortality. Most seeds fall within 1.5 m of the mother plant, resulting in a dense patch of plants that is able to displace competitors. Seed longevity in undisturbed soil is less than three years (Zamora and Thill, 1989).

C. Plants Related to Common Crupina and Their Distribution

1. Plants Related to Common Crupina

Common crupina is in the plant family Asteraceae, subfamily Cichorioideae tribe Cardueae, and subtribe Centaureinae (Susanna et al., 2006), the latter being one of five recognized subtribes. The subtribe Centaureinae is a monophyletic group (descended from a common evolutionary ancestor or ancestral group, especially one not shared with any other group) that is clearly distinct from the other subtribes in the Cardueae (Susanna et al., 2006). Whether on the basis of genetic sequence data (Susanna et al., 2006), morphological features (Garcia-Jacas et al., 2002), or isozymes (enzymes with identical function but different structure) (Garnatje et al., 2002), the two European *Crupina* species (*C. vulgaris* and *C. crupinastrum*) are unique, appearing in distinct, monophyletic groups within the subtribe Centaureinae. Only *C. vulgaris* was introduced into the United States.

The closest relatives of *C. vulgaris*, those also in the subtribe Centaureinae, include economically-important safflower (*Carthamus tinctorius*) and two natives, *Plectocephalus americanus* and *P. rothrockii* (syn. *Centaurea americana* and *C. rothrockii*, respectively). Among the introduced relatives are 34 species of *Centaurea*, many of which are weedy, and *Serratula tinctoria*, which occurs in New York and Connecticut (USDA-NRCS, 2007).

Related plants occur in the Carduineae, another subtribe of the Cardueae. Economically-important artichoke (*Cynara scolymus*) and a large number of native species in the genera *Cirsium* and *Saussurea* are members of this group. Within the genus *Cirsium* are six taxa listed either as threatened or endangered (*C. fontinale* var. *fontinale*, *C. fontinale* var. *obispoense*, *C. hydrophylum* var. *hydrophylum*, *C. loncholepis*, *C. pitcheri*, and *C. vinaceum*). Four other species of *Cirsium* are introduced. The three species of *Saussurea* co-occurring with common crupina are *S. americana, S. densa*, and *S. weberi* (USDA-NRCS, 2007). All remaining closely-related species in this subtribe are introduced (USDA-NRCS, 2007), occurring in the genera (# species) *Acroptilon* (1), *Arctium* (4), *Carduus* (5), *Cnicus* (1), *Onopordum* (3), and *Silybum* (1). None of these are commercially important.

More distantly related are species in the five remaining tribes (the Arctoteae, Cichoriae (Lactuceae), Liabeae, Mutisieae, and Vernonieae) of the subfamily Cichorioideae. Of these, there are only a few species that are native, ornamental, or agricultural in importance.

2. Distribution of Plants Related to Common

Plants in the family Asteraceae are cosmopolitan, and relatives from the tribe Cardueae are commonly distributed throughout North America. Distribution of the most closely related plants, those in the tribe Cardueae, also is cosmopolitan. Commercial production of both safflower and artichoke is centered in California, and safflower is grown commercially also in Montana. Ornamental and weedy *Centaurea* species occur in California, but ranges of both native *Plectocephalus* species occur outside the present distribution of *C. vulgaris* (USDA-NRCS, 2007). The listed *Cirsium* species occur in California, except for *C. vinaceum*, which is in New Mexico, and *C. pitcheri*, which grows near the Great Lakes (USDA-NRCS, 2007). The genus *Cirsium* contains the most native species, many of which occur within the same state as common crupina. Of the *Saussurea* species, only *S. americana* occurs within the range of common crupina (USDA-NRCS, 2007). Although many of these native or economically-important plants co-occur with common crupina at the state level, significant differences in distribution are likely, either in terms of geographic distribution or ecological niche. Most species related to common crupina are introduced or otherwise pests and therefore not of concern.

IV. Environmental Consequences

A. No Action

1. Impact of Common Crupina

a. Livestock

Although common crupina is palatable to grazing animals in the rosette stage, subsequent development of the bolt renders the plant unpalatable and results in reduction of rangeland productivity (Thill et al., 1987). Livestock avoid mature plants, preferring other plants for forage, even though common crupina does not appear to be toxic.

b. Plants

Populations of common crupina may become monocultures in infested habitat. Common crupina competes effectively with annual and perennial grasses and forbs, displacing them and native plant species.

c. Beneficial Uses

There are no beneficial, social, or recreational uses for this plant.

2. Impact from Use of Other Control Methods

The continued use of chemical, mechanical, and cultural controls at current levels would be a result if the "no action" alternative is chosen. These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *R. crupinae* to reduce common crupina populations in the contiguous United States.

a. Chemical Control

Application of herbicides can adversely affect non-target plants. Also, after chemical treatment, the site must be monitored for at least four years after the last flowering adult plants have been eliminated, and treatments repeated when necessary. Effectiveness of this and other control methods of common crupina are limited on steep slopes and in other rugged geography; these areas can be inaccessible or difficult to reach (CDFA, undated).

b. Mechanical Control

Digging, hoeing, and hand-pulling are not useful for large infestations. Removed plants must be handled carefully to avoid spreading seeds. Seeds are small and can adhere to clothing and tools and these must be cleaned before using again. Mowing as a control strategy can increase the chance of seed spread.

c. Cultural Control

Competitive grasses can suppress common crupina and effectively resist invasion. However, this type of management is difficult in remote areas.

B. Issue Permits for Environmental Release of *R. crupinae*

1. Impact of *R. crupinae* on Nontarget Plants

Host specificity of *R. crupinae* to common crupina has been demonstrated through scientific literature and host specificity testing. If the the candidate biological control agent only attacks one or a few closely related plant species, it is considered to be very host-specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

a. Scientific Literature

Ramularia crupinae was tested in France by Hasan et al. (1999). Artificial inoculation of ten species from the Asteraceae in that study resulted in disease only on common crupina (Table 1). In a complementary field study, neighboring species did not develop symptoms (Hasan et al., 1999).

Table 1. Plants inoculated with conidia of *Ramularia crupina* in a study in France (Hasan et al., 1999).

Test plant name	Tribe	RXN
	(w/in Asteraceae)	
Aster chinensis	Astereae	-
Calendula officinalis	Calenduleae	-
Carthamus tinctorius	Cynareae	-
Cynara scolymus	Cynareae	-
Gazania splendens	Arctotidae	-
Helianthus annuus	Heliantheae	-
Lactuca sativa	Lactuceae	-
Tagetes erecta	Heliantheae	-
Zinnia elegans	Heliantheae	-
Crupina vulgaris, var. *vulgaris* (as "*typica*"); ID and OR	Cynareae	+
Crupina vulgaris, var. *brachypappa*; WA	Cynareae	+
Crupina vulgaris; France	Cynareae	+

RXN=reaction, "-" = no symptoms, "+" = symptoms

b. Host Specificity Testing

Besides the testing conducted in France (Hasan et al., 1999), additional host specificity tests were conducted for approval for United States release of *R. crupinae.* Host specificity tests are tests to determine how many plant species *R. crupinae* infects, and whether nontarget plant species may be at risk.

(1) Site of Quarantine Studies

Evaluation of *R. crupinae* was conducted by the USDA, Agricultural Research Service (ARS), at the European Biological Control Lab (EBCL), Montpellier, France, and at the Foreign Disease-Weed Science Research Unit (FDWSRU), Ft. Detrick (Frederick), Maryland.

(2) Test Plant List

Test plant lists are developed by researchers for determining the host specificity of biocontrol agents of weeds in North America. Test plant lists are usually developed on the basis of phylogenetic relationships between the target weed and other plant species (Wapshere, 1974). It is generally assumed that plant species more closely related to the target weed species are at greater risk of attack than more distantly related species.

The host specificity test strategy as described by Wapshere (1974) is "a centrifugal phylogenetic testing method which involves exposing to the organism a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed." Researchers do not pursue release of biological control agents that do not demonstrate high host specificity to the target weed.

(3) Discussion of Host Specificity Testing

See appendix 2 for a description of host specificity test design.

There were no "non-target effects" caused by *R. crupinae* on any of the 29 species from the tribe Cardueae that were inoculated (Appendix 1). Among them were 11 species from the subtribe Centaureinae, including both varieties of the target and six cultivars of safflower. Among the safflower cultivars were those of commercial importance both in California and Idaho, and those high in oleic oil content. Also inoculated were 18 species from the subtribe Carduineae, including 16 species of native, North American *Cirsium* and commercially-important artichoke. Two of the *Cirsium* species that were inoculated with *R. crupinae* (fountain thistle (*C. fontinale* var. *fontinale*) and La Graciosa thistle (*C. loncholepis*)) are federally listed as endangered.

Data were further analyzed using statistical mixed models to generate Best Linear Unbiased Predictors (BLUPs). Description of the BLUP analysis and its advantages is given in Berner (2010), and application of this approach has been described in Berner and Bruckart (2012), Berner and Cavin (2012), and Berner et al. (2009). It should be noted that the BLUP output is not predictive but rather a relative measure of probability. In this case, quantitative relational data based upon certain genetic sequences of the plants were used for disease potential analysis. This enabled analysis of an additional 20 related species in the genera (number) *Carthamus* (1), *Centaurea* (7), *Carduus* (3), *Cirsium*, and five species outside of the tribe Cardueae. A total of 49 species were examined either by inoculation or by mixed model analysis, for a total of 60 taxa (species, variety, and cultivar). However, the BLUP analysis was supplemental and probably not necessary because symptoms developed only on common crupina. See Appendix 1 for more discussion of BLUP analysis.

Impacts to Safflower

Two of the four replications of host specificity testing included six cultivars of safflower (*Carthamus tinctorius*), and symptomatic individual plants were noted in one of them. Incidence and severity were very low. Of 120 plants inoculated, 82 percent were symptomless, 13 percent were rated '1', and the remaining 5 percent were rated '2' on a scale of 0–10.

This compares to an average rating of '7' for common crupina in these tests. However, identification of the pathogen revealed that symptoms were not caused by *R. crupinae* but caused by *Ramularia cynarae* (= *R. carthami*, according to Braun, 1998), a fungus clearly different morphologically from *R. crupinae*. No further study of *R. carthami* was made, and there was not further recurrence of this disease. It is believed that the source of *R. carthami* was infested seeds, but this was not investigated.

2. Impact of *R. crupinae* on Common Crupina

Preliminary studies in France suggest that *R. crupinae* causes significant damage to infected plants (Hasan et al., 1999). Of the infected plants in their study, 17 of 43 (39.5 percent) died, presumably as a result of, or in association with, infections from *R. crupinae*. Also, over the 4.5-month period the field study was run, 40 of 45 (88.9 percent) of the "uninfected" crupina control plants became symptomatic of the *Ramularia* disease. Total number of seeds was reduced by 52.1 percent in the "infected" treatment (93 from diseased plants vs. 194 from the controls).

There was no evidence that variety of common crupina (*brachyppa* or *vulgaris*) was important in terms of susceptibility or response to disease. Severe necrosis (cell injury which results in the premature death of cells in living tissue) of common crupina was observed after inoculation by *R. crupinae* (Figure 3) that included significant reduction in root growth (Table 2, Figure 3C) for data pooled by crupina variety. Change in height was also reduced after inoculation by 7.1 cm (from 39.8 to 32.7 cm for controls and inoculated plants, respectively) in one experiment. Reduction in the number of seeds per plant from disease (yield reduction of nearly 50 percent) was most evident only as the number of inoculations increased.

Table 2. Least Squares Means for seed yield and root dry weight measurements of the two varieties of *Crupina vulgaris* after a single inoculation with *Ramularia crupinae* vs. controls. Two accessions of each variety were inoculated either in the bolt or bud stages of growth, i.e., as "older" plants; shoot dry weight was included as covariate in the statistical analysis.

			Seed			
	Variety	Trt[1]	No./plant	Weight	Wt/Seed	Root dry weight
	Brachypappa (B)	C	21.9 a	0.328 a	0.016 a	0.43 a
		I	20.2 b	0.327 a	0.017 a	0.36 b
	Vulgaris (V)	C	12.4 c	0.356 a	0.030 b	0.51 a
		I	12.8 c	0.361 a	0.029 b	0.37 b
Accession	Variety					
Modoc Co., CA	B	C	26.8 a	0.42 a	0.016 a	0.349 a
		I	22.8 b	0.38 a	0.017 a	0.328 a
Lake Chelan, WA	B	C	16.8 a	0.24 a	0.016 a	0.524 a
		I	17.0 a	0.26 a	0.016 a	0.412 a
Salmon River, ID	V	C	13.2 a	0.36 a	0.030 a	0.428 a
		I	14.1 a	0.36 a	0.027 b	0.370 a
	V					
Santa Rosa, CA		C	12.5 a	0.36 a	0.030 a	0.566 a
		I	12.6 a	0.38 a	0.030 a	0.354 a

[1] Trt = Treatment; C = Control, I = Inoculated by *R. crupinae*.

Figure 3. Damage to *Crupina vulgaris* from disease caused by *Ramularia crupinae*. **A.** Non-inoculated control plant (left) and three severely diseased plants. **B.** Necrotic spots on the bolt of a diseased plant. **C.** Dried roots after a single inoculation (“treated”on the left, “controls” on the right).

3. Uncertainties Regarding the Environmental Release of *R. crupinae*

Once a biological control agent such as *R. crupina* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (common crupina) to attack nontarget plants. 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 *R. crupinae*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *R. crupinae* 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. However, significant non-target impacts on plant populations from previous releases of weed biological control agents are unusual (Suckling and Sforza, 2014).

In addition, this agent may not be successful in reducing common crupina populations in the contiguous 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 common crupina by *R. crupinae* will not be known until after release occurs and post-release monitoring has been conducted (see Appendix 3 for release protocol and post-release monitoring plan). It is expected that *R. crupinae* will reduce seed production and cause plant death, depending on appropriate environmental conditions in the field conducive to infection of common crupina. However, predicting efficacy of *R. crupinae* under field conditions after release into the environment of the United States is not possible based on the greenhouse tests conducted (Bruckart, 2013) or the study in France (Hasan et al., 1999).

4. Livestock

The reduction of common crupina by *R. crupinae* may result in improvement of pasture quality and be beneficial to livestock.

5. Plants

Based on host-specificity testing, adverse effects to native or commercially important plants are not likely from use of *R. crupinae*. In contrast, native plant species currently competing unsuccessfully with common crupina may be benefitted by release of *R. crupinae* if it is effective in reducing common crupina populations.

6. Human Health

Ramularia crupinae will have no effect on a human health. It is a plant pathogen and does not infect humans or animals.

7. Beneficial Uses

There are no known beneficial uses of common crupina, thus reduction of common crupina by release of *R. crupinae* will not affect any beneficial uses.

8. 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).

Other private and public concerns work to control common crupina in invaded areas using available chemical, cultural, and mechanical control methods. Release of *R. crupinae* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to common crupina. Effective biological control of common crupina will have beneficial effects for Federal, State, local, and private weed management programs, and may result in a long-term, non-damaging method to assist in the control of common crupina.

8. 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 and endangered species or result in the destruction or adverse modification of critical habitat.

In the contiguous United States, there are 56 plants that are federally-listed in the family Asteraceae, the same family as the target weed. Based on the host specificity of *R. crupinae* reported in testing and in the scientific literature, APHIS has determined that environmental release of *R. crupinae* may affect, but is not likely to adversely affect the plant species belonging to the Asteraceae listed in Appendix 4.

APHIS has also determined that *R. crupinae* may affect beneficially, the California tiger salamander (*Ambystoma californiense*) (Santa Barbara County, Sonoma County, and Central California Distinct Population Segments) because common crupina can dominate vernal pool areas where the salamander occurs (CDFA, 2016). Common crupina can dominate grassland and vernal pool areas, excluding native plants and lowering biodiversity. Plants that might be beneficially affected by removal of common crupina from their habitat include showy Indian clover (*Trifolium amoenum*) and Burke's goldfields (*Lasthenia burkei*) (CDFA, 2016).

A biological assessment was prepared and submitted to the U.S. Fish and Wildlife Service (FWS) and is part of the administrative record for this EA (prepared by T.A. Willard, February 2, 2017). APHIS requested concurrence from the FWS on these determinations, and received a concurrence letter dated March 19, 2019.

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 *R. crupinae* 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 are 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 *R. crupinae*.

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…."

APHIS is consulting and collaborating with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests in accordance with EO 13175.

VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *R. crupinae* on May 10, 2016. TAG members that reviewed the release petition (13-03) (Bruckart, 2013) included USDA representatives from the National Institute of Food and Agriculture, Animal and Plant Health Inspection Service, Agricultural Research Service, and U.S. Forest Service; U.S. Department of Interior's Bureau of Land Management and Bureau of Indian Affairs; Environmental Protection Agency; U.S. Army Corps of Engineers; and representatives from California Department of Food and Agriculture (National Plant Board), Mexico Secretariat of Agriculture, Livestock, Rural Development, and Fisheries, and Agriculture and Agri-Food Canada.

This EA was prepared by personnel at APHIS and ARS. The addresses of participating APHIS units, cooperators, and consultants follow.

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

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Pests, Pathogens, and Biocontrol Permits

4700 River Road, Unit 133
Riverdale, MD 20737

U.S. Department of Agriculture
Agricultural Research Service
Foreign Disease-Weed Science Research Unit
1301 Ditto Avenue
Ft. Detrick, MD 21702-5023

VII. References

Berner, D.K. 2010. BLUP, a new paradigm in host-range determination. Biological Control 53: 143–152.

Berner, D.K., and Bruckart, W.L. 2012. Comparing predictions from mixed model equations with host range determinations from historical disease evaluation data of two previously released weed biological control pathogens. Biological Control 60: 207 – 215.

Berner, D.K., and Cavin, C.A. 2012. Finalizing host range determination of a weed biological control pathogen with best linear unbiased predictors and damage assessment. BioControl 57: 235 – 246.

Berner, D.K., Bruckart, W.L., Cavin, C.A., and Michael J.L. 2009. Mixed model analysis combining disease ratings and DNA sequences to determine host range of Uromyces salsolae for biological control of Russian thistle. Biological Control 49: 68 – 76.

Braun, U. 1998. A monograph of *Cercosporella*, *Ramularia*, and allied genera (Phytopathogenic hyphomycetes). Vol. 2. IHW-Verlag, Munich.

Bruckart, W.L. 2013. Proposed field release of *Ramularia crupinae* Dianese, Hasan & Sobhian (Deuteromycotina) collected from *Crupina vulgaris* Cassini (Asteraceae) in France for biological control of common crupina, *C. vulgaris* in the United States. A Petition Submitted to the Technical Advisory Group for Biological Control Agents of Weeds. 33 pp.

California Department of Food and Agriculture. Undated. Encycloweedia: Data Sheets, Common Crupina or Bearded creeper. California Dept. Food & Agriculture.
https://www.cdfa.ca.gov/plant/IPC/encycloweedia/weedinfo/crupina.htm.
Last accessed: October 18, 2017.

California Department of Food and Agriculture. 2016. California Pest Rating. Crupina vulgaris Pers. ex. Cass.: bearded creeper. [Online].

http://blogs.cdfa.ca.gov/Section3162/?p=2241 Accessed: 19 January, 2017.

CDFA—see California Department of Food and Agriculture.

Couderc-LeVaillant, M., and Roché C.T. 1993. Evidence of multiple introductions of *Crupina vulgaris* in infestations in the western United States. Madroño. 40:63–64.

Culliney, T.W. 2005. Benefits of classical biological control for managing invasive plants. Critical Reviews in Plant Sciences. 24: 131−150.

Dianese, J., Hasan, S., and Sobhian, R. 1996. *Ramularia crupinae* sp. nov. A leaf pathogen of *Crupina vulgaris* (Asteraceae). Fitopatol. Bras. 21: 115–119.

DiTomaso, J.M., and Healy, E.A. 2007. Weeds of California and Other Western States, Vol I. Aizoaceae – Fabaceae. Publication 3488. Univ. California, Agriculture and Natural Resources, Oakland, CA.

Gamarra, R., and Roché, C.T. 2002. Distribution of the genus *Crupina* in the Iberian Peninsula and the Balearic Islands. Madroño. 49:137–142.

Garcia-Jacas, N., Garnatje, T., Susanna, A., Vilatersana, R. 2002. Tribal and subtribal delimitation and phylogeny of the Cardueae (Asteraceae): A combined nuclear and chloroplast DNA analysis. Mol. Phylogenetics and Evolution 22:51-64.

Garnatje, T., Vilaternasa, R., Roché, C.T., Garcia-Jacas, N., Susanna, A., Thill. D.C. 2002. Multiple introductions from the Iberian Peninsula are responsible for invasion of *Crupina vulgaris* in western North America. New Phytologist 154:419-428.

Hasan, S., Sobhian, R. and Knutson, L. 1999. Preliminary studies on *Ramularia crupinae* sp. nov. as a potential biological control agent for common crupina (*Crupina vulgaris*) in the USA. Ann. App. Biol. 135: 489–494.

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R., Lumbsch, H.T., Lutzoni, F., Matheny, P.B., McLaughlin, D. J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.-C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Kõljalg, U., Kurtzman, C.P., Larsson, K.-H., Lichtwardt, R., Longcore, J. Miądlikowska, J., Miller, A., Moncalvo, J.-M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schüßler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.-J., and Zhang, N. 2007. A higher-level phylogenetic classification of the fungi. Mycol. Res. 111:509-547.

Keil, D.J., Turner, C.E. 1993. Crupina. P. 245. *In:* Hickman J.C. (ed.), The Jepson Manual. Higher Plants of California. Univ. of California Press. Berkely, CA.

Louda, S.M., Pemberton, R.W., Johnson, M.T. and Follett, P.A. 2003. Nontarget effects−The Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biological control introductions. Annual Review of Entomology. 48: 365–396.

Pemberton, R.W. 2000. Predictable risk to native plants in weed biological control. Oecologia. 125: 489-494.

Prather, T.S., and Callihan, R.H. 1993. Weed eradication using Geographic Information System. Weed Technol. 7:265-269.

Roché, C. T. and Thill, D. C. 2001. Biology of common crupina and yellow starthistle, two Mediterranean winter annual invaders in western North America. Weed Science. 49:439-447.

Roché, C.T., Thill, D., and Shafii, B. 1997a. Prediction of flowering in common crupina (*Crupina vulgaris*). Weed Sci. 45:519-528.

Roché, C.T., Vilaternasa, R., Garnatje, T., Gamarra, R., Garcia-Jacas, N., Susanna, A., and Thill, D. 2003. Tracking an invader to its origins: The invasive case history of *Crupina vulgaris*. Weed Res. 43:177-189.

Stickney, P.F. 1972. *Crupina vulgaris* (Compositae, Cynareae), new to Idaho and North America. Madroño 21:402.

Suckling, D.M., and Sforza, R.F.H. 2014. What magnitude are observed non-target impacts from weed biocontrol? PLoS ONE. 9(1): e84847. doi:10.1371/journal.pone.0084847.

Susanna, A., Garcia-Jacas, N., Hidalgo, O., Vilatersana, R., and Garnatje, T. 2006. The Cardueae (Compositae) revisited: Insights from ITS, *trn*L-*trn*F, and *mat*K nuclear and chloroplast DNA analysis. Ann. Missouri Bot. Gard. 93:150-171.

TAG—see Technical Advisory Group for the Biological Control Agents of Weeds.

Technical Advisory Group for the Biological Control Agents of Weeds. 2016. Technical Advisory Group for Biological Control Agents of Weeds Manual, Interim Edition. Available: https://www.aphis.usda.gov/import_export/plants/manuals/domestic/downloads/tag-bcaw_manual.pdf. Last accessed: 27 September, 2017.

Thill, D.C., Zamora, D., Kambitsch, D. L. 1986. The germination and viability of excreted common crupina (*Crupina vulgaris*) achenes. Weed Sci. 34:237-241.

Thill, D.C., Zamora, D.L., Kidder, D. 1987. Common Crupina: Identification and Biology. Univ. Idaho, Moscow, ID. Current Information Series No. 542. 1-4.

USDA-NRCS—see U.S. Department of Agriculture, Natural Resource Conservation Service.

U.S. Department of Agriculture, Natural Resource Conservation Service. 2007. The PLANTS Database (http://plants.usda.gov). National Plant Data Team, Greensboro, NC 27401-4901 USA.

WA_NWCB. 1999. Class A Noxious Weeds, Washington State. Wash. State Noxious Weed Control Board. http://nweb.wa.gov/weed_list/Class_A_weeds.htm.

Wapshere, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Annals of Applied Biology. 77: 201–211.

Zamora, D.L., and Thill, D.C. 1989. Seed bank longevity of common crupina (*Crupina vulgaris*) in natural populations. Weed Technol. 3:166-169.

Appendix 1. Test plant species (Asteraceae) included in the host range determination for *Ramularia crupinae*. Ranked data with a value of '1' were for species that did not develop symptoms (rating of '0'). In the SAS analysis, "ties = low" was specified, so ratings of '0' were assigned a rank value of '1', which was every species except *Crupina vulgaris*. Number of individual plants tested was added to table. There were 4 separate inoculation events (repetitions), each with *Crupina vulgaris* control plants. (Bruckart, 2013)

Tribe, Subtribe					**LSMeans Ranks of**	**Mixed Model Analysis**		
Genus	**species**	**Test[1]**	**Notes**	**n**	**disease ratings**	**BLUP[2]**	**Std. Er.**	**Pr > |t|**
Cardueae, Carduinae								
Arctium	*minus*	B	Weedy			-5.64	55.27	0.54
Carduus	*nutans*	B	Weedy			-34.36	53.54	0.52
Carduus	*pycnocephalus*	I, B	Weedy	6	1 a	-38.42	52.81	0.47
Carduus	*tenuifloris*	B	Weedy			-44.40	52.88	0.40
Cirsium	*arvense*	B	Weedy			-38.80	53.71	0.47
Cirsium	*brevistylum*	I, B	Native	10	1 a	-32.53	52.93	0.54
Cirsium	*canovirens*	I	Native	15	1 a			
Cirsium	*cymosum*	I	Native	10	1 a			
Cirsium	*discolor*	I, B	Native	10	1 a	-35.67	52.90	0.50
Cirsium	*fontinale*	I, B	Native	13	1 a	-31.93	52.78	0.55
Cirsium	*fontinale* var. *obispoense*	B	Native, E/T			-33.74	52.98	0.52
Cirsium	*neomexicanum*	I, B	Native	10	1 a	-34.53	52.720	0.51
Cirsium	*occidentale*	I, B	Native	20	1 a	-34.70	52.56	0.51
Cirsium	*occidentale* var. *candidissimum*	I	Native; *C. pastoris*	11	1 a	(See *C. occidentale*)		
Cirsium	*occidentale* var. *candidissimum*	I	Native; *C. candidissimum*	4	1 a	(See *C. occidentale*)		
Cirsium	*occidentale* var. *venustum*	I	Native	10	1 a	(See *C. occidentale*)		
Cirsium	*occidentale* var. *venustum*	I	Native; *C. proteanum*	5	1 a	(See *C. occidentale*)		
Cirsium	*ochrocentrum*	I	Native	25	1 a			
Cirsium	*pitcheri*	I, B	Native, E/T	2	1 a	-37.79	52.93	0.48
Cirsium	*pumilum*	I	Native	8	1 a			
Cirsium	*scariosum* var. *citrinum*	I, B	Native, E/T; *C. loncholepis*	8	1 a	-36.93	52.88	0.49

Appendix 1 (continued)

Cirsium	*scariosum* var. *citrinum*	I	Native; *C. quercetorum*	17	1 a			
Cirsium	*rhothophilum*	I, B	Native	10	1 a	-35.29	52.76	0.50
Cirsium	*undulatum*	I	Native	1	1 a			
Cynara	*scolymus*	I, B	Commercial	20	1 a	-34.61	52.64	0.51
Picnomon	*acarna*	B	Non-native			-26.00	54.14	0.63
Saussurea	*alpina*	B	Non-native			3.63	55.24	0.95
Saussurea	*americana*	B	Native			-0.96	55.09	0.99
Saussurea	*candicans*	B	Non-native			1.87	55.04	0.97
Saussurea	*nuda*	I, B	Native	4	1 a	3.87	55.16	0.94
Serratula	*cornonata*	B	Non-native			-3.65	54.95	0.95
Silybum	*marianum*	B	Weedy			-36.64	53.71	0.50
Cardueae, Centaureinae								
Carthamus	*lanatatus*	B	Weedy			-4.32	54.10	0.94
Carthamus	*oxycanthus*	B	Weedy			11.32	52.54	0.83
Carthamus	*tinctorius*	I, B	Commercial; 6 cultivars	120	1 a[3]	11.37	52.53	0.83
Centaurea	*calcitrapa*	B	Weedy			-25.40	53.97	0.64
Centaurea	*cyanus*	B	Weedy, Ornamental			-13.60	54.21	0.80
Centaurea	*cymosum*	I, B	Weedy	1	1 a	-33.54	52.83	0.53
Centaurea	*diffusa*	B	Weedy			-28.50	52.99	0.59
Centaurea	*jacea*	B	Weedy			-20.98	53.81	0.70
Centaurea	*japonica*	I	Weedy	15	1 a			
Centaurea	*maculosa*	I, B	Weedy	10	1 a	-28.17	53.03	0.60
Centaurea	*melitensis*	I, B	Weedy	11	1 a	-31.37	52.80	0.55
Centaurea	*montana*	B	Weedy			-16.61	54.07	0.76
Centaurea	*napifolia*	B	Weedy			-13.61	53.95	0.80
Centaurea	*solsitialis*	I, B	Weedy	18	1 a	-30.16	52.67	0.57
Centaurea	*sulphurea*	B	Weedy			-12.20	54.04	0.82
Crupina	*vulgaris* (*brachypappa*)	I, B	Weedy, Target species	42	470.5 b	132.07	52.55	0.01
Crupina	*vulgaris* (*vulgaris*)	I, B	Weedy, Target species	20	464.8 b	134.43	52.49	0.01

Appendix 1 (continued)

Plectocephalus	*americanus*	I, B	Native	5	1 a	-24.76	53.14	0.64
Plectocephalus	*rothrockii*	I, B	Native	10	1 a	-27.98	52.89	0.60
Rhaponticum	*repens*	I, B	Weedy	19	1 a	-32.41	52.67	0.54
Cichorieae								
Cichorium	*intybus*	I, B	Weedy	15	1 a	-48.87	54.90	0.377
Krigia	*montana*	B	Native			-42.12	55.06	0.447
Eupitorieae								
Liatris	*spicata*	B	Native			-2.36	55.28	0.97
Astereae								
Solidago	*shortii*	B	Native			-29.08	55.09	0.60
Vernonieae								
Bacchoroides	*anthelmintica*	B	Non-native			-8.40	55.28	0.88
Callistephus	*chinensis*	B	Ornamental			-30.94	54.91	0.574
Stokesia	*laevis*	B	Native			-42.63	54.81	0.44

[1] I = inoculated, i.e., plants were inoculated by *Ramularia crupinae*; B = test of potential susceptibility made via mixed model analysis and generation of Best Linear Unbiased Predictors (BLUPs).

[2] BLUP = Best Linear Unbiased Predictor.

[3] Symptoms occurred in one of two reps on safflower in our tests, but isolation and microscopic examination of the fungus indicated the disease was caused by *Ramularia carthami* (now *R. cynarae*; see note), a pathogen of safflower that is distinctly different from *R. crupinae*. Preliminary identification was reported as *R. crupinae* (Bruckart et al., 2014), but it was found subsequently to be *R. cynarae* (= *R. carthami*, according to Braun, 1998).

Appendix 2. Host-specificity testing methods (Bruckhart, 2013)

Characterization of Plant Accessions. *Crupina vulgaris* seed were acquired from all the known infestations that occur in Idaho, Washington, and California as well as from new locations in Oregon. Variety of each accession was confirmed in this study. Growth and development of U.S. accessions of *C. vulgaris* were determined as part of this risk assessment in order to develop information about differences in behavior of each variety of the target plant in host specificity tests. In so doing, identification of the accessions by variety was confirmed, and results of vernalization studies made it possible to synchronize bolting of the two varieties and conduct comparative disease studies.

Plant stature and morphological characteristics: General descriptions of plants were made of each accession at the USDA, Agricultural Research Service, Foreign Disease-Weed Science Research Unit (FDWSRU) at Ft. Detrick, MD. Characteristics noted were plant color, general growth form, and characteristics of seed for each variety. Angle of leaf attachment was determined by trigonometry, with the leaf length serving as hypotenuse of a right triangle. Chlorophyll content of leaves was measured by optical density of an acetone extraction at both 645 and 663 λ. Total chlorophyll was calculated as: $C_{tot} = (20.2_{D645} + 8.02_{D663} \times$ the dilution factor of the preparation)/1000, thus giving the amount of chlorophyll in the preparation (mg chlorophyll/ml of prep). This was then adjusted to give the amount of chlorophyll in the leaf tissue (mg chlorophyll/g of leaf tissue). Characteristics of seed were determined for representatives of each variety. Samples of 50 seeds were weighed and measured for length. Pappus length was also recorded.

Protocol for vernalization: Plants of var. *brachypappa* take as long as a year to bolt under greenhouse conditions, a process shortened by vernalization. For this research, plants were started from seed and grown at 10 ºC with an 8-hour photoperiod. To achieve synchrony in bolting, seeds of var. *brachypappa* were started at least two weeks before those of var. *vulgaris*. Plants at similar stages of phenological development were removed after two months and used in comparative inoculation studies.

Inoculum Preparation and Plant Inoculation: The isolate of *R. crupinae* was grown on Modified Potato Carrot Agar (MPCA), a medium of 140 g each of potatoes and carrots and 20 g agar per liter of water. A mixture of conidia (asexual spores) and mycelium was harvested after 2 weeks growth at 23ºC with laboratory lighting. The spore suspension in 40 ml water was adjusted to between 10^5 and 10^6 conidia/ml for inoculation. Suspensions of inoculum that were sprayed onto plants included an unquantified number of mycelial fragments, the latter reported also capable of causing infection (Hasan et al., 1999). Although the "life cycle" of the candidate pathogen is not fully understood, plant inoculations were made under optimal conditions of plant age, inoculum concentration, dew period, and temperature. The risk assessment is considered a worst-case scenario.

Target Plant Inoculations, and Disease and Damage Measurement. There were two major focal points of this evaluation. The first was measurement of the potential for *R. crupinae* to damage common crupina. Vernalized crupina plants at different phenological stages of development were inoculated by spraying with a suspension of spores. Inoculated plants were given two 16-hour dew periods (the time from when plants were placed into the dew chamber until they were removed, a treatment that favors the pathogen and provides a good test of potential susceptibility. It is standard protocol for inoculations) at 24 ºC with an 8-hour photoperiod between. Plants were removed from the dew chambers and placed on greenhouse benches with natural light, and temperatures ranging between 25 and 30 ºC. Relative humidity was not controlled during incubation of inoculated plants in the greenhouse; it has not been an important factor in any of the many studies conducted in biological control or in other programs involving rust diseases. In some cases, plants were inoculated on two or three successive occasions. Viable seed is produced on crupina under greenhouse conditions, so seed yield data were collected along with measurements of plant height and dry weight. With development of the first seed, bolted plants were covered with mesh bags and kept for one additional month before collecting seed data. Disease ratings were also recorded, using the following rating scale: 0 = no macroscopic symptoms; 1 = 1–2% necrotic area; 2 = >2–10% necrotic area; 3 = >10–20% necrotic area; 4 = >20–40% necrotic area; and 5 = >40% necrotic area.

Two experiments were run to determine the potential for *R. crupinae* to damage common crupina. In the first, accessions representing both crupina varieties were inoculated once by the fungus. Data on number of seeds, total weight of seeds, weight per seed, and root dry weight, were collected and analyzed. There were two repetitions in this study. The second experiment involved only the two accessions of var. *brachypappa*. Among the four repetitions of this study, plants either were not inoculated (controls) or they were inoculated once, twice, or three times, at one week intervals. Seed yield data (number/plant, weight/plant, and weight/seed (calculated)), were collected and analyzed.

<u>Host Range Determination</u>. Non-target test plants were evaluated after four separate inoculations (repetitions), each with *C. vulgaris* plant as a positive control. For inoculation, non-target test plants (Table 2) were started from seed in vermiculite and transplanted to a soilless greenhouse mix. Inoculations, as described, were made when transplants were 4–6 weeks old. Representatives of 29 species in the Asteraceae in eight genera were inoculated along with common crupina. Among them were six cultivars of safflower (*Carthamus tinctorius*) and one accession of artichoke (*Cynara scolymus*), both of which are of commercial importance. Natives included 15 *Cirsium* (two representative listed species) and two *Centaurea* (*Plectocephalus*) species. Healthy plants of common crupina were included in each inoculation as a positive control to ensure conditions were favorable for disease and to provide a standard for disease response comparisons. Test plants were observed at least weekly for one month for evidence of symptoms. In the event of symptom development on a non-target test species, the plan was to put symptomatic tissue into a moist chamber to induce sporulation; fungal growth from necrotic areas would be examined microscopically after one or two weeks for fungal structures and identification of the fungus would be made. Evidence of *R. crupina* causing disease in such a case on a non-target test species would trigger initiation of additional studies designed to clarify the magnitude and meaning of disease on a non-target species.

In addition to actual inoculations, predictions about potential susceptibility were included in mixed model analyses (see below under statistical analysis) to generate Best Linear Unbiased Predictors (BLUPs) within the model established from inoculation data (Appendix 1). Description of the BLUP analysis and its advantages is given in Berner (2010), and application of this approach has been described in Berner and Bruckart (2012), Berner and Cavin (2012), and Berner et al. (2009). It should be noted that the BLUP output is not predictive but rather a relative measure of probability. In this case, quantitative relational data based upon ITS sequences were entered into the existing database and analyzed for disease potential. This enabled analysis of an additional 20 related species that were otherwise unavailable or difficult to grow. Within the Cardueae, 59.2 percent of the species in this evaluation had been inoculated, and about half of these were also subject to mixed model analysis. The plan was to obtain propagules of species identified as potentially susceptible based solely upon the BLUP output for actual inoculation, in order to determine whether it was susceptible or not under our test conditions. Plants actually tested were those that could be obtained and grown, and selection was based upon the list presented in the pre-proposal. Those tested were considered to be sufficiently representative of related species for a clear decision about a lack of risk. Unfortunately, not all species listed in the pre-proposal could be obtained or grown, or there was sufficient evidence from the results obtained to determine that additional tests were not needed.

Statistical analyses. Data were analyzed using Statistical Analysis Systems software (SAS, Cary, NC). For most data (i.e., yield, seed weight and weight per seed) analyses involved the General Linear Models (Proc GLM) procedure, with shoot dry weight data as covariate. Least square means (LSMEANS) were generated from the analysis, and differences between treatments were based on Pr > |t| for selected comparisons, based upon PDIFF output from SAS. Means were considered significantly different when $P \leq 0.05$. Seed yield data from multiple inoculation events were subject to regression analysis. Categorical rating data and mean lesion counts, each a quantitative measure of disease, were ranked using Proc Rank, and rank values were subject to GLM analysis. Ranking categorical data for analysis satisfies assumptions of statistical tests. In this case, "ties = low" was specified, so the lowest rank value ("1") was assigned to those species rated "zero" for disease. This analysis was based on data from actual inoculations. The BLUP analysis was supplemental and probably not necessary because symptoms developed only on the crupina accessions. It is a challenge to report results from the host range determination in a "middle ground" format and be transparent. Also, BLUPs are not the final answer but require ground-truthing, particularly for those species identified as potentially susceptible after analysis on the basis of sequence data.

In addition to statistical analysis of ranked rating data, disease response data from host range tests were subject also to a mixed model analysis that facilitated inclusion of quantitative relatedness of each non-target species to that of common crupina. Output of these analyses was generation of a Best Linear Unbiased Predictor, or BLUP. Description of the BLUP analysis and its advantages is given in Berner (2010), and application of this approach has been described in Berner and Bruckart (2012), Berner and Cavin (2012), and Berner et al. (2009). It should be noted that the BLUP output is not predictive but rather a relative measure of probability. As indicated previously, there is need for ground-truthing, even with the help of the mixed model analysis.

Appendix 3. Release Protocol and Post-Release Monitoring Plan for *Ramularia crupinae* (Bruckhart, 2013).

Release Protocol

Release sites will be identified and marked clearly by cooperators prior to inoculum production. Inoculum will be in the form of conidia, the asexual spore of *R. crupinae*. There are two sources of *R. crupinae*¸ each maintained under containment conditions. Some cultures are in long-term storage, and they are located already outside of the Containment Greenhouse Facility (Bldg. 374). Cultures for inoculation can be started from these, or a tube or plate of *R. crupinae* culture that has been maintained under aseptic laboratory conditions can be removed from containment after washing the outside to remove potential containment pathogens.

Once outside of containment under permit, conidia will be produced on artificial media. This can be accomplished at FDWSRU or in laboratories near release sites. Conidia will be suspended in water and plants will be inoculated by spraying a suspension of spores onto leaves. Means for providing free moisture during the infection process may be applied, such as covering plants overnight with a plastic bag.

Post-Release Monitoring

Once inoculation has taken place, each site will be monitored for symptoms of disease on crupina. This takes 2–3 weeks in the field. Plots will be monitored also for spread of the pathogen, based upon symptoms from secondary infections, and for damage to the target. Plans for damage assessments will be made before release, so that proper analysis and reporting of results is possible. Other plants in the general vicinity, particularly members of the Asteraceae, will be monitored for symptoms of disease. Preliminary surveys of diseases at each site will provide the necessary background information about potentially confusing disease scenarios. Identification of pathogens causing disease on non-target species will be attempted as part of this process.

Appendix 4. May affect determinations for listed plants in the contiguous United States.

Common Name	Determination	Impact/Effects
South Texas ambrosia	May affect, not likely to adversely affect (MANLAA)	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect South Texas ambrosia.
San Diego ambrosia	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect San Diego ambrosia or its critical habitat.
Encinitas baccharis	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Encinitas baccharis.
Sonoma sunshine	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Sonoma sunshine.
Decurrent false aster	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect decurrent false aster.
Florida brickell-bush	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Florida brickell-bush or its designated critical habitat.
Cape Sable thoroughwort	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that

		included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Cape Sable thoroughwort or its designated critical habitat.
Florida golden aster	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Florida golden aster.
Fountain thistle	MANLAA	The closest relatives of common crupina are in the tribe Cardueae, subtribe Centaureinae. Fountain thistle (*Cirsium*) occurs in the sub tribe Carduineae, the other subtribe of the Cardueae, and thus would be at highest risk of listed plants in being affected by *R. crupinae*. Many *Cirsium* species are considered sympatric with common crupina. However, in host specificity testing, 18 species and subspecies, no symptoms of *R. crupinae* developed on any *Cirsium* species tested, or any other species tested besides common crupina. Therefore, environmental release of *R. crupinae* may affect, but is not likely to adversely affect Fountain thistle.
Chorro Creek bog thistle	MANLAA	The closest relatives of common crupina are in the tribe Cardueae, subtribe Centaureinae. Chorro Creek bog thistle (*Cirsium*) occurs in the sub tribe Carduineae, the other subtribe of the Cardueae, and thus would be at highest risk of listed plants in being affected by *R. crupinae*. Many *Cirsium* species are considered sympatric with common crupina. However, in host specificity testing, 18 species and subspecies, no symptoms of *R. crupinae* developed on any *Cirsium* species tested, or any other species tested besides common crupina. Therefore, environmental release of *R. crupinae* may affect, but is not likely to adversely affect Chorro Creek bog thistle.
Suisun thistle	MANLAA	The closest relatives of common crupina are in the tribe Cardueae, subtribe Centaureinae. Suisun thistle (*Cirsium*) occurs in the sub tribe Carduineae, the other subtribe of the Cardueae, and thus would be at highest risk of listed plants in being affected by *R. crupinae*. Many *Cirsium* species are considered sympatric with common crupina. However, in host specificity testing, 18 species and subspecies, no symptoms of *R. crupinae* developed on any *Cirsium* species tested, or any other species tested besides common crupina. Therefore, environmental release of *R. crupinae* may affect, but is not likely to adversely affect Suisun thistle or its critical habitat.
La Graciosa thistle	MANLAA	The closest relatives of common crupina are in the tribe Cardueae, subtribe Centaureinae. La Graciosa thistle (*Cirsium*) occurs in the sub tribe Carduineae, the other subtribe of the Cardueae, and thus would be at highest risk of listed plants in being affected by *R. crupinae*. Many *Cirsium* species are considered sympatric with common crupina. However, in host specificity testing, 18 species and subspecies, no symptoms of *R. crupinae* developed on any *Cirsium* species tested, or any other species tested besides common crupina. Therefore, environmental release of *R. crupinae* may affect, but is not likely to adversely affect La Graciosa thistle or its critical habitat.

Pitcher's thistle	MANLAA	The closest relatives of common crupina are in the tribe Cardueae, subtribe Centaureinae. Pitcher's thistle (*Cirsium*) occurs in the sub tribe Carduineae, the other subtribe of the Cardueae, and thus would be at highest risk of listed plants in being affected by *R. crupinae*. Many *Cirsium* species are considered sympatric with common crupina. However, in host specificity testing, 18 species and subspecies, no symptoms of *R. crupinae* developed on any *Cirsium* species tested, or any other species tested besides common crupina. Pitcher's thistle was directly tested in host specificity testing. Therefore, environmental release of *R. crupinae* may affect, but is not likely to adversely affect Pitcher's thistle.
Sacramento Mountains thistle	MANLAA	The closest relatives of common crupina are in the tribe Cardueae, subtribe Centaureinae. Sacramento Mountains thistle (*Cirsium*) occurs in the sub tribe Carduineae, the other subtribe of the Cardueae, and thus would be at highest risk of listed plants in being affected by *R. crupinae*. Many *Cirsium* species are considered sympatric with common crupina. However, in host specificity testing, 18 species and subspecies, no symptoms of *R. crupinae* developed on any *Cirsium* species tested, or any other species tested besides common crupina. Therefore, environmental release of *R. crupinae* may affect, but is not likely to adversely affect Sacramento Mountains thistle.
Otay tarplant	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Otay tarplant or its designated critical habitat.
Gaviota tarplant	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Gaviota tarplant or its designated critical habitat.
Smooth coneflower	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect smooth coneflower.
Ash Meadows sunray	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Ash Meadows sunray or its designated critical habitat.

Willamette daisy	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Willamette daisy or its designated critical habitat.
Parish's daisy	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Parish's daisy or its designated critical habitat.
Zuni fleabane	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Zuni fleabane.
San Mateo woolly sunflower	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect San Mateo woolly sunflower.
Ash Meadows gumplant	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Ash Meadows gumplant or its designated critical habitat.
Virginia sneezeweed	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Virginia sneezeweed.
Pecos sunflower	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may

		affect, but is not likely to adversely affect Pecos sunflower or its designated critical habitat.
Schweinitz's sunflower	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Schweinitz's sunflower.
Whorled sunflower	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect whorled sunflower or its designated critical habitat.
Santa Cruz tarplant	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Santa Cruz tarplant or its designated critical habitat.
Lakeside daisy	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect lakeside daisy.
Texas prairie dawn-flower	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Texas prairie dawn-flower.
Burke's goldfields	MANLAA, Beneficial	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Burke's goldfields. Removal of common crupina could benefit Burke's goldfields because the plant can dominate vernal pool areas where this species occurs (CDFA, 2016). Thus, *R. crupinae* may affect beneficially Burke's goldfields.
Contra Costa goldfields	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as

		common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Contra Costa goldfields or its designated critical habitat.
Beach layia	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect beach layia.
San Francisco lessingia	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect San Francisco lessingia.
Heller's blazingstar	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Heller's blazingstar.
Scrub blazingstar	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect scrub blazingstar.
Santa Cruz malacothrix	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Santa Cruz malacothrix.
Island malacothrix	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect island malacothrix.
Mohr's Barbara buttons	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants

		tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Mohr's Barbara buttons..
San Joaquin wooly-threads	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect San Joaquin wooly-threads.
San Francisco Peaks ragwort	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect San Francisco Peaks ragwort or its designated critical habitat.
White-rayed pentachaeta	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect white-rayed pentachaeta.
Lyon's pentachaeta	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Lyon's pentachaeta.
Ruth's golden aster	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Ruth's golden aster.
Hartweg's golden sunburst	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Hartweg's golden sunburst.
San Joaquin adobe sunburst	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may

		affect, but is not likely to adversely affect San Joaquin adobe sunburst.
Layne's butterweed	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Layne's butterweed.
Houghton's goldenrod	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Houghton's goldenrod.
Short's goldenrod	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Short's goldenrod.
Blue Ridge goldenrod	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Blue Ridge goldenrod.
Malheur wire-lettuce	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Malheur wire-lettuce or its designated critical habitat.
California taraxacum	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect California taraxacum or its designated critical habitat.
Ashy dogweed	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect ashy dogweed.

Last Chance townsendia	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect Last Chance townsendia.
Big-leaved crownbeard	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect big-leaved crownbeard.
Desert yellowhead	MANLAA	*Ramularia crupinae* could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as common crupina (Asteraceae). However, in host specificity tests that included the most closely related plants to common crupina, no plants tested exhibited symptoms or supported development of *R. crupinae*. Therefore, APHIS has determined that release of *R. crupinae* may affect, but is not likely to adversely affect desert yellowhead.
Showy Indian clover	MA-Beneficial	Common crupina can dominate grassland and vernal pool areas, excluding native plants and lowering biodiversity. Rare taxa that might be affected include grassland species such as showy Indian clover (*Trifolium amoenum*) (CDFA, 2016). Removal of common crupina from the habitat of this plant may be beneficial to it.