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Risk Assessment and Implications of Common Crupina Rust Disease for Biological Control

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Evaluation of *Puccinia crupinae*, the causal agent of a rust disease on common crupina (*Crupina vulgaris*), for biological control is described. Susceptibility of accessions of common crupina that represent both varieties of the target from the five populations in the United States indicate that the disease has potential to control common crupina, but differences were noted between accessions on the basis of pustule count, yield (i.e., number and weight of achenes per plant), and shoot dry weight data after multiple inoculations. One accession from Modoc, CA, was not affected in greenhouse tests and would likely not be affected in the field if a permit to release *P. crupinae* were granted. None of the nontarget species of 26 taxa from the tribes Cardueae and Cichoriae were symptomatic, so the pathogen is likely safe to use in North America.

Nomenclature: Common crupina, *Crupina vulgaris* Pers. ex Cass. var. *brachypappa* P. Beauv.; *C. vulgaris* var. *vulgaris* Pers. ex Cass.

Key words: Asteraceae, *Crupina vulgaris*, invasive plant, plant disease, Pucciniales, rust fungus.

Biological control of common crupina (*Crupina vulgaris* Pers. ex Cass.; CRVU2 [USDA, NRCS 2014], CJNVU [EPPO or Bayer Code]) by plant pathogens is under investigation. Common crupina is listed as a Federal noxious weed and bears similar stature in 11 states not known presently to have infestations (USDA, NRCS 2014). Common crupina competes with grasses and forbs in grazing and natural areas of the western United States, thus potentially reducing density of desirable forage plants and overall usefulness of infested pastures and rangelands (Miller and Thill 1983). Infestations of common crupina are isolated and limited to five main locations in the United States. Each population is relatively small compared to other invasive species. Even so, there is considerable concern about this plant where it occurs and where it might occur. Currently, there is a single large infestation in Idaho, one location in Washington State, two locations in California, and a regional infestation in northeastern Oregon (Bruckart et al. 2014; Roché et al. 2003; USDA, NRCS 2014).

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Although common crupina is a single species in the United States, there are two varieties, *C. v.* var. *vulgaris* (formerly referred to as "var. *typica*") and *C. v.* var. *brachypappa* (Couderc-LeVaillant and Roché 1993). The varieties differ both morphologically and biologically (Bruckart et al. 2014; Roché et al. 1997; Roché and Thill 2001). None of the infestations in the United States is a mixture of these varieties, and within each infestation, the variety remains true morphologically and biologically. Differences in varietal response to leaf detachment experiments and to disease by *Ramularia crupinae* Dianese, Hasan and Sobhian did not occur in artificial greenhouse studies (Bruckart et al. 2014).

The present distribution of common crupina is limited in the United States, but reductions in forage productivity and livestock carrying capacity have been reported where high densities occur (Miller and Thill 1983). The potential for spread is also considerable (Patterson and Mortensen 1985). Infestations of common crupina are cryptic, particularly at low population densities, because the common crupina plant is thin and very fine in stature, making it very difficult to see in a mixture of rangeland and pasture plants (Gamarra and Roché 2002; Roché et al. 2003). For this reason, the known distribution of common crupina in the United States might be considerably larger than described. Conventional weed management approaches, although effective, are often impractical for common crupina because of infestation size, terrain, or ecological sensitivity (Prather and Callihan 1993;

Management Implications

Common crupina is an invasive plant of ranges and pastures in the states of California, Idaho, Oregon, and Washington. Because there are no other effective or practical management strategies, focus has been on developing an obligate rust fungus from Greece for biological control. In the United States, there are five distinct populations, representing two varieties of common crupina, var. *brachypappa* and var. *vulgaris*, that differ in morphology and in biology. Evaluation of a rust disease, caused by *Puccinia crupinae*, was made on representatives of each population and both varieties from all infestations of common crupina in the United States. Disease from *P. crupinae* did not develop in tests of 26 nontarget relatives in the Asteraceae, Tribes Cardueae and Cichoriae. The strain of *P. crupinae* under evaluation is thus considered host-specific. Data on dew temperature suggest the pathogen will establish and cause disease on common crupina in the field, if permit for release is granted. Also, severe disease developed under greenhouse conditions on all but the accession from Modoc, CA, and measurable damage occurred to at least one accession from Lake Chelan, WA, in multiple inoculation studies. Implications from this suggest that the accession from Modoc, CA, would not be adversely affected in the field by the strain of *P. crupinae* in this study. The rust disease would likely infect and potentially damage common crupina in the majority of its range in the United States, if released. Results also suggest need for at least one additional strain of *P. crupinae*, or another candidate biological control agent, to bring pressure on all populations of common crupina in the United States.

http://www.co.chelan.wa.us/nw/special_projects/common_crupina.htm). For these reasons, there is significant concern about this pest and justification for its status as "noxious."

The possibility for biological control has been proposed by Hasan et al. (1999) and Bruckart et al. (2014) in studies with *R. crupinae*, and inclusion of biological control candidates in weed management programs is of interest where common crupina occurs (CDFA n.d.). At present there is no pathogen or arthropod agent available, although a petition for introduction of *R. crupinae* for biological control is in review (Bruckart et al. 2014). *Puccinia crupinae* Ranoj. (Basidiomycota, Pucciniomycetes, Pucciniales) is an autoecious rust fungus that has not been previously evaluated for potential in biological control of common crupina.

An assessment of *P. crupinae* for biological control of common crupina in the United States is described in this paper, and three aspects are considered in this evaluation: (1) importance of variety and population (accession) of common crupina in susceptibility to disease by *P. crupinae*, (2) measures of damage caused by the pathogen on accessions of common crupina, and (3) a host range determination as a measure of potential risk (or safety). As part of the risk assessment, the hypothesis was also tested that variety and accession within variety do not differ in susceptibility to disease by *P. crupinae*.

Materials and Methods

All research was conducted in a BSL-3 containment greenhouse and laboratory, because both the target plant and the pathogen are regulated organisms. Common crupina is listed as a Federal Noxious Weed (USDA, APHIS 2015). Also, *P. crupinae* was collected originally in Europe and, as such, must be contained until approved for use in the United States.

Plant Accessions and Management. In this study, experiments included two common crupina accessions of var. *brachypappa* from Lake Chelan, WA (Chelan), and Modoc, CA (Modoc), and four accessions of var. *vulgaris*, from Salmon River, ID (Idaho), Santa Rosa, CA (Ste. Rosa), and Wallowa and Umatilla counties in northeastern Oregon. Accessions from Oregon were collected within 130 km (80.8 mi) of each other in a region where common crupina is widespread. For this reason, they were considered as a single population (NE Oregon) during data analysis. Details for source and molecular information about these accessions are given in Table 1. DNA regions ITS1, 5.8S, and ITS2, were sequenced for each accession as described in Bruckart et al. (2014) and deposited in GenBank (Table 1).

All accessions of common crupina were started as seed, germinated at 10 C (50 F) in vermiculite, and transplanted at the cotyledon stage into 10-cm- (3.9-in-) diam pots filled with a standard artificial growth medium of peat (41%), bark (11%), perlite (23%), vermiculite (23%), sand (2%), and Micromax trace minerals (Scotts Miracle-Gro, Marysville, OH 43041). Following transplanting, plants were grown at 10 C with an 8-h photoperiod in order to bring the two varieties into reproductive phenological synchrony. Vernalization was necessary because the varieties otherwise differ in the initiation of reproductive phenology (Roché et al. 1997), and a vernalization protocol adapted for these studies facilitated comparisons between varieties at similar stages of development (Bruckart et al. 2014). Plants were vernalized for 4 to 6 wk prior to inoculation. In order to get synchrony for the start of reproductive phenology, accessions of var. *brachypappa* were subject to a 2-wk longer vernalization period.

Inoculum Preparation, Plant Inoculation, and Disease and Damage Measurement. *Puccinia crupinae* isolate FDWSRU 01-056 was used in this study. It was collected by D. Berner between Daria and Kozani, from the region of West Macedonia in Greece (40.3745°N, 22.064°E) on May 31, 2001. Specimen of the isolate was deposited at the USDA, ARS, Systematic Mycology and Microbiology Laboratory herbarium (BPI 880889), and sequence of the ITS1, 5.8S, ITS2 DNA region, was deposited into the GenBank database (HQ184334).

Urediniospore inoculum was increased on the Chelan accession and used either fresh or after ultra-cold (−80 C)

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Table 1. Varieties and accessions of *Crupina vulgaris* from the United States studied, and LSMean for the number of pustules on the three most-diseased leaves (Pust3) after inoculation by *Puccinia crupinae*.

Variety	Accession[a]	FDWSRU no.[b]	GenBank no.[c]	Latitude	Longitude	Pust3[d,e]
C. v. brachypappa	Modoc, CA	CJNVU 45	KC768347	41.343	−120.911	1.6 a
C. v. brachypappa	Lake Chelan, WA	CJNVU 43	KC768345	47.838	−120.024	24.3 b
C. v. vulgaris	Santa Rosa, CA	CJNVU 17	HM921416	45.893	−116.337	11.2 b
C. v. vulgaris	Salmon River, ID	CJNVU 41	KC768344	38.425	−122.649	17.9 b
C. v. vulgaris	Wallowa Co., OR	CJNVU 46	KC768348	45.325	−116.824	nt[f]
C. v. vulgaris	Umatilla Co., OR	CJNVU-47	KC768349	45.846	−118.384	nt

[a] Source locations of accessions.
[b] Accession number at the USDA, ARS, Foreign Disease—Weed Science Research Unit.
[c] Sequence for ITS1, 5.8S, and ITS2 DNA.
[d] Numbers followed by the same letter are not significantly different, $P \leq 0.05$.
[e] Back-transformed data from ln analysis.
[f] Abbreviation: nt, not tested.

storage. Both common crupina and nontarget test plants were inoculated by spraying an aqueous suspension of urediniospores via atomizer over healthy specimens. Inoculum was applied at the rate of 0.5 mg (0.00002 oz) urediniospores per plant.

Following inoculation, plants received two 16-h dew periods with an 8-h photoperiod between them. The dew treatment was given in the dark at 18 to 21 C, except in the case of a dew temperature study. Plants were removed from dew chambers, placed on greenhouse benches, and maintained between 21 and 25 C during disease development. Plants in the greenhouse were subject to natural light supplemented when needed by 1,000-W metal halide lamps to give a minimum 14-h photoperiod.

Susceptibility of Accessions. Based upon differential response by accession to rust disease following inoculations of nonvernalized plants, a more detailed inoculation study was done. Vernalized plants of four accessions, two each of each variety synchronized for the initiation of reproductive phenology, were inoculated following the protocol described. They were monitored, and when flower color was first noted in the experiment, all individual plants were covered with cheesecloth bags in order to capture seeds. One month after this time, experiments were terminated and seed counts were made. Pustule counts were also recorded for the three most-diseased leaves (Pust3), i.e., those leaves that were at the optimum stage of susceptibility. There were four repetitions over time, and means from each repetition were used in statistical analyses.

Dew Temperature Study. A dew temperature study was conducted to determine optimal conditions for disease for accessions from each U.S. infestation. Based upon preliminary tests that included dew periods of 4, 8, 12, and 16 hr, a dew period of 12 hr was selected as best for comparison of accessions. Vernalized plants were inoculated as described and subjected to dew temperatures ranging from 12 to 22 C. Plants were benched after a single dew treatment and observed for symptom development, as described. Pustules were counted on the three most-infected leaves, and the mean number of pustules per leaf from seven inoculations (repetitions) was used for analysis.

Damage from Multiple Inoculations. Damage to common crupina was measured in a study involving multiple inoculations by *P. crupinae*. Plants from each of four accessions, synchronized for reproductive phenology, were either not inoculated (controls) or were inoculated as described, once, twice, or three times, at 7- to 10-d intervals. The standard dew treatment protocol was used, as described. Reproductive phenology developed during this period, and when the first flower color was noted, individual plants were covered in cheesecloth bags to capture seeds. The experiment was terminated 1 mo after bagging and data were collected on yield, i.e., seed number per plant (SeedNo), seed weight per plant (SeedWt), weight per seed (WtPerSd, calculated), and shoot dry weight (ShDWT).

Host Range Determination. The protocol described for inoculation of common crupina was used also to test nontarget plant susceptibility to *P. crupinae*. Seed of test plants were germinated in vermiculite, and seedlings were transplanted into the greenhouse mix and grown at 25 C. Inoculations were made when transplants were 4 to 6 wk old. Three repetitions of nontarget test plant inoculations were made over time. Each repetition included common crupina as a positive control. Complement of nontarget species varied among repetitions because of seed availability and quality. Also, the total number of plants inoculated varied among species for the same reasons. Noninoculated control plants of test species were included for comparison.

The host range determination included only representative species in the Asteraceae most closely related to

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Table 2. Mixed model analysis output from multiple-inoculations (trt) of *Crupina vulgaris* accessions (acsn), representing two varieties (vty), by *Puccinia crupinae*.

			Variable			
			Ln_SeedNo[a]	Ln_ShDWT[b]	Ln_SeedWt[c]	Ln_WtPerSd[d]
Effect	Variety		Pr > |t|	Pr > |t|	Pr > |t|	Pr > t|
vty			0.20	0.25	<0.01	< 0.001
acsn (vty)			0.04	0.24	0.04	0.82
trt(acsn*vty)			< 0.01	0.01	0.04	0.73
	acsn[e]	vty[f]	Estimate[g]			
Intercept			3.62**	0.784**	0.877**	0.034**
vty		B	−0.188	0.117	−0.382**	−0.013**
vty		V	0	0	0	0
acsn(vty)	Modoc	B	0.236*	−0.107	0.132	0.001
acsn(vty)	Chelan	B	0	0	0	0
acsn(vty)	Salmon R	V	−0.352	0.001	−0.233	−0.002
acsn(vty)	Ste. Rosa	V	−0.225	0	−0.164*	−0.002
acsn(vty)	NE Oregon	V	0	nt[h]	0	0
trt(acsn*vty)	Modoc	B	−0.069	−0.016	−0.044	−0.001
trt(acsn*vty)	Salmon R	V	−0.095	−0.052	−0.020	0.001
trt(acsn*vty)	Chelan	B	−0.141**	−0.073**	−0.072**	−0.001
trt(acsn*vty)	Ste. Rosa	V	−0.043	−0.079*	−0.001	0.001
trt(acsn*vty)	NE Oregon	V	−0.084	Nt	0.050	0.001
AIC			54.9	−20.7	−24.4	−611.7

[a] Natural log of the number of seeds per plant (SeedNo).
[b] Natural log of the mean shoot dry weight per plant (ShDWT).
[c] Natural log of the mean weight of seeds per plant (SeedWt).
[d] Natural log of the calculated mean weight per seed (WtPerSd).
[e] Accession designation by source location: Modoc, CA; Lake Chelan, WA; Salmon River, ID; Santa (Ste.) Rosa, CA; and northeastern (NE) Oregon (Umatilla County and Wallowa County accessions, pooled data).
[f] Variety of *Crupina vulgaris*: B, var. *brachypappa*; V, var. *vulgaris*.
[g] Pr > |t| indicated by: *, P < 0.05, **, P < 0.01.
[h] Abbreviation: nt, not tested.

common crupina. A total of 26 nontarget taxa, i.e., species, varieties, and cultivars, in seven genera were inoculated with *P. crupinae* (Table 2). Specifically tested were six cultivars of *Carthamus tinctorius* L. (safflower), commercially-important *Cynara scolymus* L. (artichoke), six native *Cirsium* spp., six introduced *Centaurea* spp., and the natives *Plectocephalus* (*Centaurea*) *rothrockii* (Greenm.) D.J.N. Hind.(Rothrock's knapweed) and *Saussurea nuda* Ledeb. D. C. Eaton (American saw-wort). Two genera of plants from the Tribe Cichoriae were also tested, including the natives *Agoseris grandiflora* (Nutt.) Greene (bigflower agoseris), *A. retrorsa* (Benth.) Greene (spearleaf agoseris), and *Malacothrix glabrata* (A. Gray ex D. C. Eaton) A. Gray (smooth desertdandelion), and *Microseris elegans* Greene ex A. Gray (elegant silverpuffs), and the exotic species, *M. saxatilis* (Nutt.) Torr. and Gray (cliff desertdandelion).

Test plants were examined regularly, and final data on symptom development were collected 1 mo after inoculation. A plus-and-minus rating scheme was used to record and evaluate nontarget responses, because no macroscopic symptoms were observed on any nontarget species.

Statistical Analyses. Data were analyzed using Statistical Analysis Systems software (SAS, Cary, NC; ver. 9.2). Datasets were tested using Proc Univariate for normality of residuals, and the (ln + Constant) transformation was applied, if necessary, prior to analysis to satisfy assumptions. Pust3 data were analyzed using Proc GLM, and separation of LSMeans was on the basis of PDIFF output; means were considered significantly different if: $P > |t| \leq 0.05$. Dew temperature data were analyzed also by Proc GLM, from which regression equations were calculated.

Data from the multiple-inoculation study were analyzed using Proc GLIMMIX with replication as a random variable in each analysis. Natural log-transformed data for SeedNo, SeedWt, WtPerSd, and ShDWT, were evaluated.

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Results and Discussion

Common crupina is unique as a target for biological control, and factors that make it unique are also important considerations in evaluations of candidate biological control agents. Most significant is the known variability of common crupina in the United States. The two varieties differ in morphology (Bruckart et al. 2014; Couderc-LeVaillant and Roché 1993), biology (Roché et al. 1997), isozymes (Garnatje et al. 1998), and RAPDs (Roché et al. 2003). This variability is the result of four, or possibly five, independent introductions into the United States, according to Roché et al. (2003), who provided evidence for at least two introductions per variety. Results from the present study suggest that accessions of var. *vulgaris* are similar in response to infection by *P. crupinae*, even though they might be the result of two or three separate introductions (Roché et al. 2003). In contrast, accessions of var. *brachypappa*, which are likely the result of separate introduction events (Roché et al. 2003), represent extremes in susceptibility to disease by *P. crupinae*.

The first indication of variability in disease response within common crupina occurred after inoculation of four nonvernalized accessions representing the two varieties. Extreme response to disease was noted between the varieties (Figure 1). Plants of var. *brachypappa* (Figure 1A and 1C) developed pustules typical of a compatible rust disease reaction, i.e., pustules were large, dark brown, and full of friable urediniospores. There was some necrosis where pustules were dense, particularly on the Modoc accession (Figure 1A), but otherwise very little chlorosis of plant tissue was observed. Significantly more pustules developed on the Chelan accession of var. *brachypappa* (Figure 1C) than on the accession from Modoc (Figure 1A). In contrast, both accessions of var. *vulgaris* developed only a few pustules, which created an hypersensitive response that killed symptomatic leaves (Figure 1B and 1D).

The hypersensitive response noted for var. *vulgaris* did not occur after plants had been vernalized, but differences among accessions were noted. Analysis of Pust3 data, i.e., the average number of pustules per leaf on the three most-diseased leaves, were significantly lower on the Modoc accession than were counts from three other accessions (Table 1). Although Pust3 results for Chelan (var. *brachypappa*) and the two var. *vulgaris* accessions were not significantly different, pustule counts for the Chelan accession were between 1.4 and 2.2 times greater than average of Pust3 data for var. *vulgaris* (Table 1).

Similar differences were noted also in the dew temperature study that also included var. *vulgaris* from northeastern Oregon (Figure 2). Curves for var. *brachypappa* represented extremes in disease response over the range of temperatures. The accession from Modoc developed very low levels of disease at all temperatures, compared to the response by the Chelan accession. This latter accession was characterized

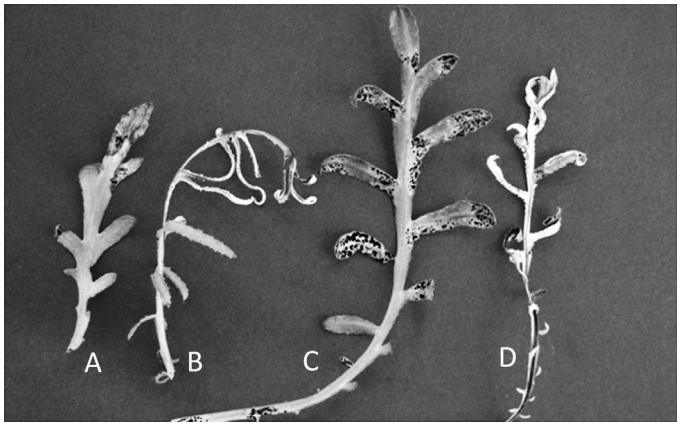

Figure 1. Signs of the fungus (pustules) and symptoms of disease (stunting and wilting) on leaves of four *Crupina vulgaris* accessions artificially inoculated by *Puccinia crupinae* (left to right: location, variety): (A) Modoc, CA, *brachypappa*; (B) Salmon River, ID, *vulgaris*; (C) Lake Chelan, WA, *brachypappa*; and (D) Santa Rosa (Sonoma Co.), CA, *vulgaris*.

by limited disease at the cooler and warmer extremes of treatments and the greatest disease of all accessions at the optimum, near 18 C. Response curves for var. *vulgaris* were similar but flatter than that of the Chelan accession. Regression curves suggest also that there is relatively more disease for var. *vulgaris* at the cooler and warmer extremes of the study than occurred with the Chelan accession. Regardless, optimal conditions for disease in this study were similar to those of other rust diseases, including *Puccinia chondrillina* Bubák & Syd. vs. rush skeletonweed, *Chondrilla juncea* L. (Emge et al. 1981) and *Puccinia jaceae* Otth vs. yellow starthistle, *Centaurea solstitialis* L. (Bennett et al. 1991).

Common crupina is facultatively self-compatible (Roché and Thill 2001), which enabled studies on seed yield in greenhouse tests. In the multiple-inoculation study, seed yield as affected by disease was studied. In the statistical analysis, treatment nested within accession nested within variety, i.e., trt(acsn*vty), was significant for SeedNo, SeedWt, and ShDWT, but not for WtPerSd (Table 2). Slopes were negative for the variables SeedNo, SeedWt, and ShDWT. This suggests that seed yield was lower as the number of inoculations increased, but only slopes for data from the Chelan accession were significantly different from zero. Greater effect of disease on the Chelan accession is consistent with results from other parts of this study.

Disease caused by *P. crupinae* is limited to common crupina and the fungus is considered host specific. This conclusion is based on the lack of symptoms on nontarget test plants (Table 3). The genus *Crupina* is in the Tribe Cardueae, subtribe Centaureinae (Garcia-Jacas et al. 2001; Susanna et al. 2006), which includes species of *Carthamus* and *Centaurea*. Common crupina is unique within the

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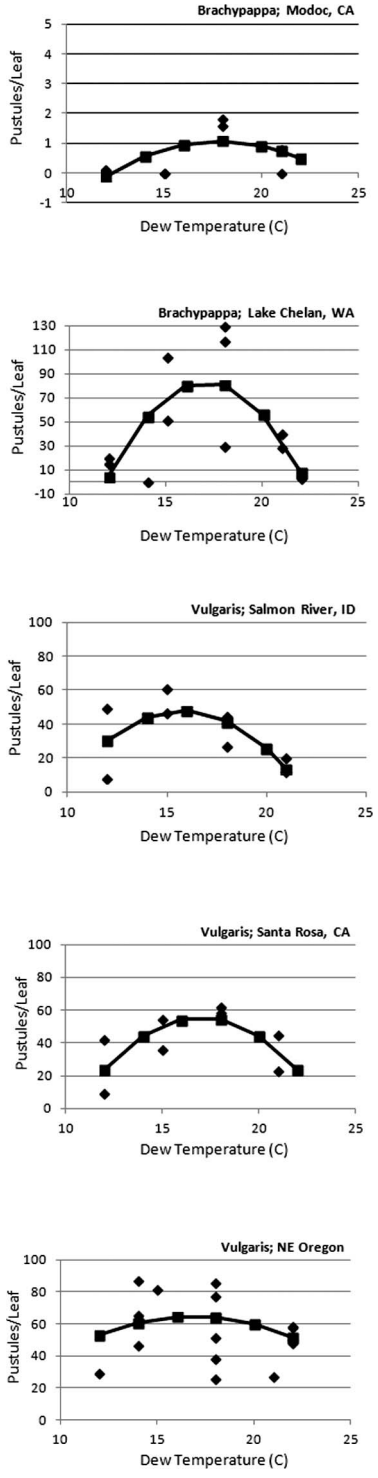

Figure 2. Regressions, based on analysis of ln mean number of seeds (back-transformed for graphing) vs. temperature during a 12-hr dew period after inoculation of *Crupina vulgaris* with *Puccinia crupinae*. Populations from five locations and two varieties occurring in the United States are represented. Equations describing each curve are: for Modoc, $y = -9.92 + 1.23x - 0.0344x^2$ ($R^2 = 0.357$); for Lake Chelan, $y = -817.2 + 105.7x - 3.1x^2$ ($R^2 = 0.480$); for Salmon River, $y = -260.6 + 39.2x - 1.245x^2$ ($R^2 = 0.502$); for Santa Rosa, $y = -311.6 + 43.2x - 1.27x^2$ ($R^2 = 0.519$); and for northeastern Oregon (Umatilla and Wallowa counties, pooled), $y = -76.3 + 16.8x - 0.5x^2$ ($R^2 = 0.086$). Mean values are indicated by filled diamonds.

subtribe. Garcia-Jacas et al. (2001) were unable to associate *Crupina* species with any particular group in the subtribe. In an earlier study using isozymes, Garnatje et al. (1998) showed that of two accessions of common crupina, one from Washington (now var. *brachypappa*) and the other from Idaho (now var. *vulgaris*) were in a clade distinct from species of *Cheirolophus*, *Centaurea*, and *Serratula*, and that the two accessions from the United States were distinct from a French accession of *C. vulgaris*.

Understanding the target plant is fundamental to success in biological control. Three examples are provided. *Puccinia chondrillina* was considered a success in the biological control of rush skeletonweed despite the existence of plant forms that were resistant to the isolate introduced into Australia (Burdon et al. 1981). A similar scenario was identified in the United States by Emge et al. (1981) that has yet to be resolved. Evaluation of *Prospodium tuberculatum* "(Speg.) Arthur" for biological control of *Lantana camara* L. revealed differential susceptibility among several forms of the target, leading Thomas et al. (2006) to conclude that the genetic diversity within the *L. camara* complex to be a limiting factor for management by a classical biological control agent. Russian thistle is also now known to be a complex of *Salsola* species (Hrusa and Gaskin 2008). It was originally considered to be a single species, but evidence by Ryan and Ayres (2000) revealed the presence of cryptic, genetically divergent populations, which were found to differ in susceptibility to *Colletotrichum gloeosporioides* "(Penz.) Penz. & Sacc.", under evaluation for biological control in the United States (Bruckart et al. 2004).

Results from the present study suggest that *P. crupinae* has considerable potential for biological control of *C. vulgaris* in the United States, despite identification of a resistant population. It is host-specific and there is evidence that the Chelan accession, and possibly those of var. *vulgaris*, is damaged under conditions of the study. Considering the differential response to disease by *P. crupinae* in this study and evidence about the Spanish origins of populations in the United States (Roché et al. 2003), additional exploration for isolates of *P. crupinae*, or other candidates for biological control, would be justified. Common crupina is a winter annual that flowers between mid-May and June (Roché et al. 1997). As an annual, elimination of seed production would result in control of the plant. Agents that affect seed production should be sought.

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Table 3. Results of host range determination of *Puccinia crupinae*.

Tribe	Genus	Species	Acsn or Cv[a]	*n*[b]	Disease[c]	Status[d]
Cardueae	*Crupina*	*vulgaris*	A	20	+	I
	Crupina	*vulgaris*	B	20	+	I
	Crupina	*vulgaris*	C	70	+	I
	Crupina	*vulgaris*	D	20	+	I
	Cirsium	*brevistylum* Cronq.		10	−	N
	Cirsium	*cymosum* (Greene) J. T. Howell.		10	−	N
	Cirsium	*fontinale* (Greene) Jeps.		10	−	N
	Cirsium	*neomexicanum* A. Gray		10	−	N
	Cirsium	*occidentale* (Nutt.) Jeps.		10	−	N
	Cirsium	*occidentale* (Nutt.) Jeps. var. *venustum* (Greene) Jeps.		10	−	N
	Centaurea	*calcitrapa* L.		5	−	I
	Centaurea	*diffusa* Lam.		2	−	I
	Centaurea	*melitensis* L.		10	−	I
	Centaurea	*napifolia* L.		5	−	I
	Centaurea	*solstitialis*		15	−	I
	Centaurea	*stoebe* L. subsp. *micranthos* (Gugler) Hayek auct. non Lam.		10	−	I
	Cynara	*scolymus*		10	−	C
	Carthamus	*tinctorius*	Birdseed	10	−	C
	Carthamus	*tinctorius*	Finch	10	−	C
	Carthamus	*tinctorius*	Montola 2000	10	−	C
	Carthamus	*tinctorius*	S-345	10	−	C
	Carthamus	*tinctorius*	88-OL	10	−	C
	Carthamus	*tinctorius*	99-OL	10	−	C
	Plectocephalus	*rothrockii*		10	−	N
	Saussurea	*nuda*		1	−	N
Cichoriae	*Malacothrix*	*glabrata*		3	−	N
	Malacothrix	*saxatalis*		4	−	N
	Microseris	*elegans*		5	−	I
	Agoseris	*grandiflora*		5	−	N
	Agoseris	*retrose*		5	−	N

[a] Abbreviations: Acsn, plant accession; Cv, cultivar.

[b] *n*, number of plants inoculated. Note: inoculations were made in four repetitions. The complement of plants tested varied between repetitions on the basis of available seed and viability (quality) of seed.

[c] Plants were either symptomatic, i.e., diseased (+), or not (−).

[d] Plant species: C, cultivated; I, introduced; N, native (N).

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