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Assessment of *Boeremia exigua* var. *rhapontica*, as a biological control agent of Russian knapweed (*Rhaponticum repens*)

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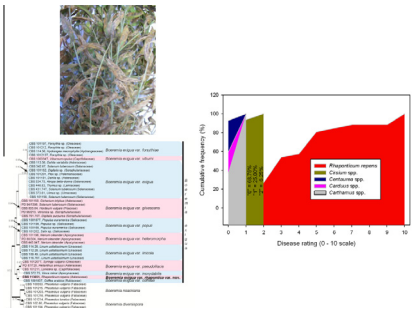
HIGHLIGHTS

- *Boeremia exigua* var. *rhapontica* was found to be a unique genetic entity.
- Disease caused by *Boeremia ex. rhapontica* was specific to *Rhaponticum* spp.
- Above-ground damage to *R. repens* was nearly twice that for other species.

GRAPHICAL ABSTRACT



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ABSTRACT

Russian knapweed (*Rhaponticum repens* (L.) Hidalgo) is an herbaceous perennial weed that was introduced and has become invasive in the United States, particularly in the semi-arid west. It is characterized by its extensive root system, low seed production, and persistence. The weed has caused serious reductions in yields and crop value and may significantly devalue the land itself. Conventional control strategies have been inadequate because of the size of infestations and economic and environmental costs of control. Biological control has been a sought-after potential solution to this weed problem. In the summer of 2002, diseased *R. repens* plants were collected near Cankiri, Turkey, and the facultative saprophytic fungus *Boeremia exigua* isolate FDWSRU 02-059 was isolated from diseased plants. Bayesian analysis of the actin, beta-tubulin, calmodulin, elongation factor, and ITS genes, of 66 isolates, representing the ten species of *Boeremia* and the 11 varieties of *B. exigua*, including FDWSRU 02-059, showed that the isolate is a unique genetic entity and was named *B. exigua* var. *rhapontica* Berner, Woudenberg & Tunali, var. nov. MycoBank MB809363. Disease incidence and severity data from host-range determination tests conducted at 25 °C, the optimum temperature for growth and sporulation of *B. ex. rhapontica*, with adequate dew periods, were combined with a genetic distance matrix based on ITS sequences of 66 plant species related to *R. repens*. The combined disease and genetic data were analyzed by mixed model equations to produce best linear unbiased predictors (BLUPs), standard errors, and $P > |t|$ values, in *t*-tests against zero, for disease incidence and severity for each species. BLUPs of disease incidence were significantly different from zero only for three *Rhaponticum* spp. while BLUPs of disease severity rankings were significantly different from zero only for *R. repens*, *Rhaponticum carthamoides*, *Rhaponticum uniflorum*, and *Leuzea*

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berardioides. Best linear unbiased predictors for differences in above-ground dry weights between control and inoculated plants of a subset of the species evaluated were not significant. However, above-ground damage by *B. ex. rhapontica* to *R. repens* was nearly twice that for any other species, except *Rhaponticum* species.

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1. Introduction

Russian knapweed (*Rhaponticum repens* (L.) Hidalgo) is an herbaceous perennial of the Asteraceae family that propagates by seeds and vegetative means. Its natural range extends from Turkey throughout Central Asia to China and Mongolia (Carpenter and Murray, 1998). Russian knapweed has become widespread in the United States and Canada, particularly in the semi-arid west. It is more competitive than other weedy species in occupying disturbed areas (Maddox et al., 1985). Both in its native and exotic range, initial colonization of a site by Russian knapweed involves establishment of genets from seeds or from small root fragments, but subsequent population development seems to occur almost exclusively by the production of shoots via clonal growth (Bottoms et al., 2001). It is characterized by its extensive root system, low seed production, and persistence (Watson, 1980). It contains an allelopathic polyacetylene compound which inhibits the growth of competing plants (Watson, 1980; Stevens, 1986).

On agricultural land, Russian knapweed has caused serious reductions in yields and crop value, and it may even significantly devalue the land itself (Watson, 1980). Russian knapweed is poisonous to horses and can cause a neurological disorder called "chewing disease" or "nigropallidal encephalomalacia" (Allred and Lee, 1999; Cordy, 1978). The symptoms resemble those of Parkinson's disease in humans and are characterized by an acute inability of the animal to eat or drink (Robles et al., 1997). Infestations of Russian knapweed can survive indefinitely through their root system (Watson, 1980). A stand in Saskatchewan has survived for almost 100 years (Allred and Lee, 1999), and Watson (1980) reported that stands of Russian knapweed have been reported to survive for more than 75 years. Native perennial grass species are frequently driven out by Russian knapweed infestations (Carpenter and Murray, 1998; Rice et al., 1992).

Conventional control strategies have been inadequate because of the size of infestations, economic and environmental costs of chemical control, and the relatively low monetary return from grazing and recreational land use (Carpenter and Murray, 1998). According to Carpenter and Murray (1998), sustainable control requires integration of mechanical, chemical, and biological controls and proper land management. As with other creeping perennials, the key to controlling Russian knapweed is to stress the weed and cause it to expend root reserves (Beck, 1998). Most recommendations for control of Russian knapweed are based on chemical control practices developed in North America. The standby herbicide options have been picloram, clopyralid, clopyralid plus 2,4-D, metsulfuron, and glyphosate (Beck, 1998; Duncan, 1994; Whitson, 2001).

Biological control of *R. repens* is a sought-after alternative and has been pursued with a nematode *Subanguina picridis* (=*Paranguina picridis*, =*Mesoanguina picridis*), a gall wasp *Aulacida acroptilonica*, a gall midge *Jappiella ivannikovi*, and an accidentally introduced rust fungus *Puccinia acroptili* (Ceasar-ThonThat et al., 1995; Kovalev et al., 1975; Rosenthal et al., 1993; USDA, APHIS, 2008, 2009; Watson, 1973, 1986). None of these have reduced populations sufficiently to restore land to its original uses.

Only nine species of fungi have been reported as causing disease on *R. repens* (Farr and Rossman, 2012). Among these is an isolate of *Phoma exigua* (now *Boeremia exigua*, Aveskamp et al., 2010) from *R. repens* in Turkey that is the subject of this study. The isolate of *B. exigua* (Desm.) Aveskamp, Gruyter & Verkley (FDWSRU isolate 02-059) reported on in this study was collected in Turkey in 2002 (Tunali et al., 2003). This isolate is a facultative saprophyte that is typically parasitic on *R. repens* but can grow and sporulate on dead vegetation and artificial media (Agrios, 2005). Infection of *R. repens* by this fungus results from germinating conidia, chlamydospores, and mycelia that form appressoria and infection pegs that directly penetrate the leaf epidermis and form intercellular haustoria that invaginate host cells, utilize cell contents, and produce phytotoxins. Infection results in leaf blight characterized by irregular, charcoal-colored, necrotic lesions at the leaf tips and margins, and frequently, necrotic whole leaves and plants (Tunali et al., 2003, Fig. 1). The fungal hyphae grow intercellularly, after infection, through plant tissues, and the fungus becomes necrotrophic. Disease progresses acropetally, from site of infection, by phytotoxin production and translocation and subsequent colonization by the fungus of toxin-affected tissue. The teleomorph (sexual stage) of this variety has never been observed *in vivo* or *in vitro*. FDWSRU 02-059 is the only known isolate, and it is a candidate biological control agent for *R. repens* in the U.S., pending the outcome of host range determination tests and approval for release.

For most plant pathogen candidates for biological weed control, clear conclusions about host specificity based upon host-range determination tests are difficult to achieve. This is in large part due to the nature of plant disease, which is the physiological manifestation of a three-way interaction among a susceptible host (a plant that can become diseased), a virulent pathogen (a parasite that can cause disease), and a favorable environment for disease development (Agrios, 2005). Pathogens produce many virulence factors, and plants have a corresponding range of defense factors (Agrios, 2005). Interactions among these factors and different environments frequently result in some disease manifestation, particularly among closely related plants, but low levels of disease or

Fig. 1. Symptoms of *Boeremia exigua* var. *rhapontica* on *Rhaponticum repens* after artificial inoculation in a field at Ayas, Turkey.

disease-like symptoms, i.e., only a few pustules or lesions on a few plants or plant parts, do not typically indicate susceptibility of the plant to the disease-causing organism. The low levels of disease or disease-like symptoms do not usually progress further because of plant defense factors. Consequently improvements in methods for accurate disease assessment have been sought since the establishment of the science in the late 1800s. Agrios (2005) and Madden et al. (2007) summarize these assessment methods and variability in disease expression. These summaries clearly illustrate that the induction and assessment of disease in host-range determination tests are unique processes that are not the equivalent of "no-choice" tests with arthropods – pathogens do not make choices, and plant disease is the result of complex interactions.

However, in all host-range determination tests among non-target plant species other important considerations arise. One of the most serious of these considerations is the validity of inferences on reactions of non-target species based on limited tests. Host-range tests are typically conducted in greenhouses or, infrequently, in small field plots, and statistical tests, typically descriptive statistics, are performed to determine whether disease or damage to the species tested is significant. But, regardless of the statistical outcome, the results pertain only to the infinitesimally small sub-sample of the plants of the species tested and not to the species as a whole. A better approach, to more closely predict the reactions of species, and address other considerations in host-range determination is evaluation of species' reactions through Mixed Model Equations (MME) that integrate genetic relationships among species with disease reaction data.

The approach is based upon Henderson's mixed model equations (MME) (Henderson, 1975, 1977) and the generation of Best Linear Unbiased Predictors (BLUPs). The MME are a quantitative genetics tool that enables prediction of disease responses of plant species by combining molecular genetics data with disease incidence and severity data from host range tests. The advantages of the MME for analysis of host range data have been described and discussed for three pathogens, including two facultative saprophytes (Berner and Cavin, 2012; Berner, 2010; Berner et al., 2009a,b; Bruckart et al., 2014), and the approach has been validated with historical host range data of another two pathogens (Berner and Bruckart, 2012).

The objectives of this study were to: (1) determine the genetic relatedness of FDWSRU 02-059 to other *Boeremia* spp. and varieties and (2) use the MME to determine the host range of isolate 02-059 for its safety to release as a classical biological control agent of *R. repens* in the U.S.

2. Materials and methods

2.1. Population of biological control agent

One isolate from *R. repens*, from Turkey, has been successfully established in containment. This isolate (FDWSRU 02-059) is the subject of all evaluations described. The isolate has been deposited in the U.S. National Fungus Collection in Beltsville, MD as BPI 843350 and in the CBS – KNAW Fungal Biodiversity Centre (CBS) in Utrecht, The Netherlands as CBS 113651. DNA sequences of the ITS 1, 5.8S, and ITS2 regions (ITS) have been deposited in GenBank of the National Center for Biotechnology Information as AY367351. Axenic cultures were produced from an initial single hyphal-tip transfer from the original isolation.

2.1.1. Genetic relatedness of FDWSRU 02-059 to other Boeremia species and varieties

The actin (ACT), β-tubulin (BTUB), calmodulin (CAL), translation elongation factor 1-α (TEF1) genes, and the ITS were sequenced for 66 isolates, representing the 10 species of *Boeremia* and the 11 varieties of *B. exigua* (Aveskamp et al., 2009), with *Phoma herbarum* as outgroup (Fig. 2). The isolates were obtained from the collection of the CBS – KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. DNA was extracted using the UltraClean Microbial DNA Isolation Kit (Mobio laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The ITS and BTUB genes were sequenced as described by Woudenberg et al. (2009) using the primer pairs V9G (de Hoog et al., 1998)/ITS4 (White et al., 1990), and BT2Fw/BT4Rd (Woudenberg et al., 2009). The ACT and CAL genes were sequenced as described by Damm et al. (2012), using the primer pair ACT-512F/ACT-783R and CAL-228F/CAL-737R (Carbone and Kohn, 1999). The TEF1 gene was sequenced as described by Woudenberg et al. (2013) using the primer pair EF-728F/EF-986R (Carbone and Kohn, 1999). Bionumerics v. 4.61 (Applied Maths, St-Martens-Latem, Belgium) was used to compute consensus sequences from both forward and reverse sequences. Multiple sequence alignments were generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html), and adjusted by eye when necessary.

A Bayesian 50% majority rule consensus tree was constructed with Mr. Bayes v. 3.2.1. (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), with the temperature set at 0.1, a sample frequency of 100, and stopped when the average deviation of split frequencies dropped below 0.01 with the burn-in set at 25%. The best nucleotide substitution model for each partition was determined with Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). For ACT, BTUB and TEF1 a GTR model was suggested, with gamma distributed rate for ACT and TEF1, for CAL a TrN model with gamma distributed rate, and for ITS a HKY model.

2.2. Host-range determination

2.2.1. Plant species

All host-range and damage assessment tests were conducted in the quarantine facility of the USDA, ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, Maryland, USA. Three species of *Rhaponticum* and the closely related species *Leuzea berardioides* were analyzed for susceptibility to FDWSRU 02-059 along with another 62 species of related plants (Hidalgo et al., 2006). These included species in the Asteraceae family and subfamilies Carduoideae and Cichorioideae. Tribes analyzed included Cardueae, Cichorieae, Heliantheae, and Vernonieae, and subtribes included Carduinae, Centaureinae, Helianthinae, Chichorinae, Vernonininae. The list of 53 species that were inoculated, which constitute the basic list of species closely related to the target weed as proposed by Wapshere (1974), and their ITS sequences, are given in Table 1. Thirteen species that were not inoculated but were included in the MME analyses are indicated by "NT" in the seed lot/plant source column of Table 1. Their ITS sequences are also indicated. Plant names are in accordance with the PLANTS database (USDA, NRCS, 2011). In every repetition of the host-range tests, susceptible Russian knapweed plants were included as positive (susceptible) controls. This insured that conditions were adequate for infection and provided a standard with which to measure any non-target host plant reactions.

2.2.2. Plant inoculations

Most seed acquisitions of *Rhaponticum* and related species were collected from the field by experts knowledgeable about current identification of *Rhaponticum* and related species in the U.S. Some collections were purchased from commercial sources or acquired from plant introduction stations. Non-target test plants for the host range determination were assembled from a number of different sources (Table 1). All plants were grown from seeds.

Fig. 2. Bayesian 50% majority rule consensus tree of 66 *Boeremia* species and *B. exigua* varieties based on the ITS, ACT, BTUB, CAL, and TEF1 DNA sequences. Strain numbers and host are indicated following species/varieties name. Bayesian posterior probabilities (PP) are given at the nodes, thickened lines indicate a PP of 1.00. The tree was rooted to *Phoma herbarum* (CBS 615.75). The new variety *B. ex. rhapontica* is highlighted.

To maintain virulence, *R. repens* plants inoculated with conidia of FDWSRU 02-059 were grown in cubicles within quarantine in isolation from other plants. Insects in the cubicles were routinely controlled with insecticide. After disease development, FDWSRU 02-059 was re-isolated and cultured from the plants in the cubicles. Conidia from cultures of these re-isolations were used in subsequent cycles (inoculations) of host-range testing.

Seedlings were inoculated 4–6 weeks after planting, by spraying a suspension of conidia at the rate of 10^6 conidia per ml water with polysorbate 20 wetting agent (0.125% v/v). All plants were sprayed with the inoculum suspension until runoff. Inoculated plants were given a dew treatment of 18 h at 25 °C. Plants were removed from dew, incubated in a greenhouse at 21–25 °C under natural light that was artificially supplemented to give a 16 h photoperiod, and observed for symptom development. That is, all tests were run under conditions that were optimal for disease.

2.2.3. Disease assessment

Plants were observed and rated weekly for disease development for 8 weeks after each inoculation (repetition), and disease severity ratings from each plant (sample) within each repetition on the eighth week were used for analysis in this study. The following 0–10 disease severity rating scale was used: 0 = no macroscopic symptoms, 1 = 1–10% diseased plant tissue, 2 = 11–20% diseased plant tissue, …, 10 = 91–100% diseased plant tissue. Plants of *R. repens* were included as a positive check in each repetition. Each repetition was a separate inoculation in time. An average of 23.6 ± 4.28 plants per species were distributed in an average of 3.2 ± 0.50 repetitions per species. Disease reactions of the averages of plants within repetitions were analyzed. An additional 51.5 ± 4.98 (average plus/minus standard error) repetitions per species were generated from the relationship matrix among species (Section 2.3). The number of plants tested in each repetition

Table 1
Plant species tested for disease incidence and severity caused by *Boeremia exigua* var. *rhapontica*, plant sources, source of DNA (ITS) sequences[a], and GenBank accession numbers.

Genus species and authority	Seed lot, plant source	ITS sequence source	GenBank accession
Achillea millefolium L.	ACHMI-1, PI 26884, WRPIS[b]	GenBank	AY603185
Arctium minus (Hill) Bernh.	ARCMI-1[c], Herbiseed	FDWSRU	HM921426
Baccharoides anthelmintica (L.) Moench	BACAN-1, PI 304909	FDWSRU	HM009327
Callistephus chinensis (L.) Nees	CALCH-12, PI 599231, WRPIS[b]	FDWSRU	HM921407
Carduus nutans L.	CRUNU-13, Ft. Detrick, MD	FDWSRU	HQ540426
Carduus pycnocephalus L.	CRUPY-7, CDFA[d]-Putah Creek Preserve- Solano Co., CA	FDWSRU	EF123105
Carduus tenuiflorus Curtis	CATEN-3, CDFA #387	FDWSRU	HM921408
Carthamus glaucus M. Bieb.	CAUGL2-2, PI 243151	FDWSRU	HQ407425
Carthamus lanatus L.	CAULA-7, PI 202728 WRPIS	FDWSRU	HM921409
Carthamus oxyacantha M. Bieb.	CAROX-1, PI 426500 NCRPIS	FDWSRU	HM009326
Carthamus palaestinus Eig	CAPAL-2, PI 235663 WRPIS	FDWSRU	HQ407426
Carthamus tenuis (Boiss. & Blanche) Bornm.	CAUGL-2, PI 244354 WRPIS	GenBank	GU969646
Carthamus tinctorius L.	CAUTI-19, Kimberlee Thompson, CAL/WEST Seeds-	FDWSRU	HM921410
Centaurea calcitrapa L.	CENCA-2, CECAL-19, #00163, Solano Co, CA	FDWSRU	HM009325
Centaurea cyanus L.	CENCY-10, Ferry-Morse 8352	FDWSRU	HQ407427
Centaurea diffusa Lam.	CENDI-1, Yakima County, Yakima, WA. Collectors: G. Piper/D. Whaley	FDWSRU	HM009323
Centaurea melitensis L.	CENME-4, Tulare County, CA.	FDWSRU	HQ540425
Centaurea x *moncktonii* C. E. Britton	CENDT-2, Kittitas County, WA. Collector: C. Roché	FDWSRU	JF728989
Centaurea montana L.	CENMO-3, Park Seed, Holland Lot#AEM7	GenBank	GU969636
Centaurea napifolia L.	CENNA-1, Thouraya Souissi, INAT, Tunisia	FDWSRU	HM921412
Centaurea solstitialis L.	CENSO-21, CDFA #378	FDWSRU	HQ218999
Centaurea stoebe L. subsp. micranthos (Gugler) Hayek	CENMA-10, Syracuse, NY (Huckle)	FDWSRU	FJ969855
Centaurea sulphurea Willd.	CENSU-1, CESUL-1, 00116, Sacramento, CA	FDWSRU	HM009322
Cichorium intybus L.	CICIN-6, PI 503599 NCRPIS[e]	FDWSRU	HM921413
Cirsium arvense (L.) Scop.	CIRAR, Ft. Detrick, MD	FDWSRU	HM921414
Cirsium fontinale (Greene) Jeps. var. *fontinale*	CIRFO-4, Churro Creak Bog. San Luis Obispo Co., CA	GenBank	AF443695
Cirsium fontinale var. *obispoense*	NT[f]	GenBank	AF443696
Cirsium hydrophilum (Greene) Jeps. var. *hydrophilum*	NT	GenBank	AF443698
Cirsium occidentale (Nutt.) Jeps. var. *venustum* (Greene) Jeps.	CIROCVE-2, Rancho Santa Ana Botanic Gardens	GenBank	AF443702
Cirsium pitcheri (Torr. ex Eaton) Torr. & A. Gray	CIRPI-3, John K. Morton, University of Waterloo, Canada	FDWSRU	HM009328
Cirsium rhothophilum S.F. Blake	CIRRH-2, CDFA-Mike Pitcairn	GenBank	AF443709
Cirsium scariosum var. *citrinum*	CIRLO-4, CDFA-Mike Pitcairn	FDWSRU	HQ407428
Cirsium vulgare (Savi) Ten.	CIRVU-5, Thouraya Souissi, INAT, Tunisia	GenBank	AF443715
Crupina vulgaris Cass.	CJNVU-17, Accession 'B', Salmon River, ID	FDWSRU	HM921416
Cynara scolymus L.	CYUSC-3, Early Violet, MACRO seeds	GenBank	AJ404744
Erigeron annuus (L.) Pers.	ERIAN-1, B&T World Seeds	GenBank	AF118489
Erigeron clokeyi Cronquist	ERICL-1, PI 30226, WRPIS	FDWSRU	HM921425
Erigeron eatonii A. Gray	ERIEA-1, PI 30824, WRPIS	FDWSRU	HM009324
Erigeron rhizomatus Cronquist	NT	GenBank	AF046992
Helianthus annuus L.	HELAN-9, PI 468637, NCRPIS	GenBank	AF047927
Helianthus eggertii Small	HELEG-1, PI 27676, NCRPIS	GenBank	AF047962
Helianthus schweinitzii Torr. & A. Gray	NT	GenBank	AF047964
Hieracium albiflorum Hook.	HIEALB-1, PI 230526, WRPIS	GenBank	AJ633418
Krigia montana (Michx.) Nutt.	NT	GenBank	HQ172903
Lactuca sativa L.	LACSA-1, Ed Hume Seeds	GenBank	AY504693
Leuzea berardioides = *Rhaponticum berardioides* (Batt.) Hidalgo	NT	GenBank	DQ310948
Liatris spicata (L.) Willd.	LISP-1, Prairie Moon Nursery	FDWSRU	HM921417
Linum usitatissimum L.	LIUUT-2, PI 522273 NCRPIS	GenBank	EU307117
Onopordum acanthium L.	ONRAC-1, CDFA 222	GenBank	AY914827
Picnomon acarna (L.) Cass.	NT	GenBank	AY826311
Plectocephalus americanus (Nuttall) D. Don in R. Sweet	CENAM-2, CEAME-2, Desert Botanical Garden, Phoenix, AZ	FDWSRU	HM921411
Plectocephalus rothrockii (Greenman) D. J. N. Hind	CEROT-5, Lot A7 Hardplants.com Apple Valley, MN	FDWSRU	FJ969854
Rhaponticum carthamoides (Willd.) Iljin	RHACA-11, PI 390005, NCRPIS	GenBank	DQ310933
Rhaponticum repens (L.) Hidalgo	ACRRE-7, Colorado State University	FDWSRU	HM009320
Rhaponticum uniflorum (L.) DC.	NT	GenBank	DQ310932
Saussurea alpina (L.) DC.	NT	GenBank	JN808237
Saussurea americana D.C. Eaton	NT	FDWSRU	HM921418
Saussurea candicans C. B. Clarke	NT	GenBank	JN808239
Saussurea nuda Ledeb.	SAUNU-1, PI 204521 WRPIS	FDWSRU	HM921423
Serratula coronata L.	NT	GenBank	AY826327
Silybum marianum (L.) Gaertn.	SYLMA-2, CDFA #386	FDWSRU	HM921420
Solidago shortii Torr. & A. Gray	NT	GenBank	AY523854
Stokesia laevis (Hill) Greene	STLA6-4, PI 537297, OPGC[g]	GenBank	EF155800
Taraxacum officinale F. H. Wigg.	TAROF-1,	GenBank	AY548211
Vernonia missurica Raf.	VENMI-1, AMES 26009, NCRPIS	FDWSRU	HM921422
Vernonia noveboracensis (L.) Michx.	VENNO-2, AMES 24586, NCRPIS	FDWSRU	HM921421

[a] Source of ITS sequence only.
[b] USDA, ARS, Western Regional Plant Introduction Station, Pullman, WA.
[c] Bayer code abbreviations followed by number are FDWSRU seed lot numbers.
[d] California Department of Food and Agriculture.
[e] USDA, ARS, North Central Regional Plant Introduction Station.
[f] Not tested for disease reaction.
[g] Ohio State University, Ornamental Plant Germplasm Center.

and the number of repetitions depended on availability of plant material and relative importance of the species in specificity tests. Disease data for species for which there were no disease severity ratings were represented as missing values (".") in the dataset.

2.2.4. Damage assessment

Damage to individual plants of a subset of 19 species in each repetition of the host range determination study was assessed eight weeks after inoculation with FDWSRU 02-059. Damage was assessed by first obtaining dry weights of above-ground plant parts by harvesting individual plants at the soil line, oven-drying at 100 °C for 24 h, and then weighing the dried materials. Because growth rates and plant architecture differed among species, it was necessary to compensate for these differences by adjusting dry weights for inoculated plants of the same species in the same repetition. Adjusted weights were obtained by averaging the dry weights of the control plants of each species in each repetition and subtracting weights of individual plants of the same species in the same repetition from the control average. The averages of these differences for each species in each repetition were analyzed. An additional 16 species, for which damage data was not obtained but which were included in the genetic distance matrix, were included in the MME analyses. Eight of the 13 species listed as not tested in Table 1 were included in the analyses as were *Callistephus chinensis*, *Carthamus oxyacantha*, *Centaurea montana*, *Cichorium intybus*, *Cirsium pitcheri*, *Crupina vulgaris*, *Helianthus eggertii*, and *Rhaponticum carthamoides*. Damage was not assessed on plants of these latter species because there was inadequate plant material for testing. All 35 species analyzed for damage are listed in Table 4.

2.3. Analyses of disease and damage among species

Fifty three plant species were inoculated with *B. ex. rhapontica* and evaluated for disease severity and incidence in replicated greenhouse tests. By incorporating a genetic distance matrix into the model, an additional 13 species, that could not be grown and evaluated because of inability to obtain seeds or failure of seed germination, were also evaluated. Ordinal data from the rating scales for disease severity were ranked, using the Rank procedure of the Statistical Analysis System (SAS, SAS Institute Inc., 2004). The variable that was ranked was the disease severity rating for each plant within each species and repetition. The mean and variance for each repetition and species was computed from the ranks of disease severity among the plants (Shah and Madden, 2004), and the means of ranks from each repetition were analyzed. A graphic summary of disease severity results was prepared. To calculate disease incidence for additional analysis, any diseased plant within a repetition was assigned a value of 1, and non-diseased plants were assigned a value of 0. The sum of these values within each repetition was then divided by the total number of plants tested in each repetition to form a proportion of diseased plants in each repetition. Proportions of 0 and 1 were set to 0.01 and 0.99, respectively. These proportions were then converted to logit values, that map binomial values between proportions of 0 and 1 onto the real expected probability (Schabenberger and Pierce, 2002), for each species in each repetition: $\text{logit} = \ln(\text{proportion}/(1 - \text{proportion}))$. These logit values for disease incidence were subsequently analyzed. For each species analyzed, ITS sequences were either generated at the Foreign Disease-Weed Science Research Unit (FDWSRU) of USDA, ARS or obtained from GenBank (Table 1). A distance matrix among species was developed using methods described in Berner et al. (2009a,b). ITS sequences of the species were aligned with the ClustalW2 tool (Larkin et al., 2007), and the output alignment file was then analyzed by quartet puzzling, with TREE-PUZZLE software (Schmidt et al., 2002), to generate both a matrix of pairwise maximum likelihood distances among species and a quartet puzzling tree of relationships among species. *Vernonia noveboracensis* was used as the outlier in the analysis. The distance matrix output from TREE-PUZZLE was used in the subsequent predictive analyses, and a file of maximum likelihood branch lengths, generated from the distance matrix, was read into TreeViewX software v. 0.5.0 (Page, 1996) to draw a cladogram among species. Ranks of disease severity ratings and logit values for disease incidence for each repetition of each species were combined with a relationship matrix generated from the genetic distance matrix (Berner et al., 2009a,b) in a SAS program (SAS Institute Inc., 2004).

Differences in oven-dry weights between non-inoculated plants of 19 species and inoculated plants of the same species, i.e., species-specific damage attributable to FDWSRU 02-059, were analyzed in the same manner as disease severity and incidence data (Berner, 2010). An additional 16 species with no damage data were also incorporated into the analyses. The disease reaction, damage data, and genetic distance data were analyzed, within SAS, as mixed model equations (MME) in which the variance–covariance structure was completely specified by the genetic distance matrix and the variance among species for the dependent variable, i.e., differences in above-ground dry weights between non-inoculated and inoculated plants.

3. Results

3.1. Population of biological control agent

Because the amplification/sequencing of the BTUB region of CBS 101151, the CAL region of CBS 760.73, CBS 489.94 and CBS 101206 and the TEF1 region of CBS 109.79, CBS 118.93, CBS 760.73, CBS 100354 and PD 88/304 failed, these genes were included as missing data in the combined analysis. The aligned sequences of the ITS (464), BTUB (247), ACT (230), CAL (491) and TEF1 (303) gene regions of the 66 strains had a total length of 1735 characters, with respectively 22, 39, 55, 110 and 171 unique site patterns. After discarding the burn-in, the 50% majority rule consensus tree was calculated based on 10,352 trees (Fig. 2).

With the exception of *B. ex.* var. *lilacis*, the *B. ex.* varieties are separated from the 9 other *Boeremia* species. The clustering of *B. ex.* var. *lilacis* outside the *B. exigua* cluster was already noted by Aveskamp et al. (2009). Our isolate of interest, FDWSRU 02-059, clusters within the exigua complex close to *B. exigua* var. *coffeae*, which is originally found on coffee plants (*Coffea Arabica*, Rubiaceae). Based on the molecular and biological data presented here, we propose the new variety, *B. exigua* var. *rhapontica*, for our pathogen of *R. repens*.

3.1.1. Description of B. ex. rhapontica

B. exigua var. *rhapontica* Berner, Woudenb. & Tunali, var. nov. MycoBank MB809363.

Etymology: varietal name refers to the host genus on which it occurs, *Rhaponticum*.

Colonies on OA 6.0 cm diameter after 7 days, regular, white to light salmon with white aerial mycelium. Colonies on MEA 7.0 cm diameter after 7 days, regular, white to tan/olivaceous with white/olivaceous aerial mycelium. Colonies on CA 5.0 cm diameter after 7 days, regular, white/olivaceous to olivaceous with olivaceous aerial mycelium. Application of NaOH did not result in a color change in cultures.

Conidiomata pycnidial, scattered to aggregated, light brown to black; globose, subglobose, irregular, 50.3–199.1 μm (average 126.59 μm) in diameter, with 1–2 ostioles, ostioles papillate at the tip of necks up to 80 μm long.

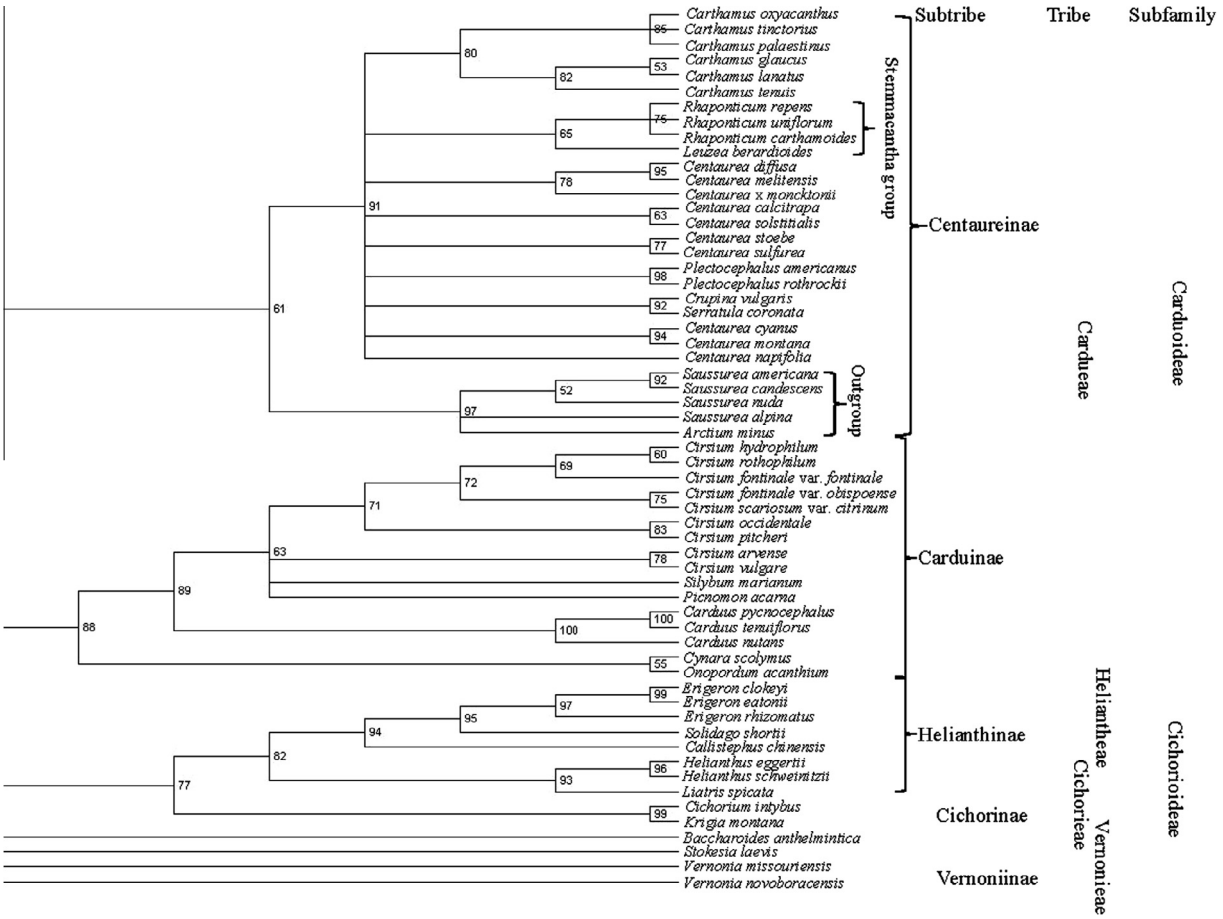

Fig. 3. Cladogram of species closely related to *Rhaponticum repens* based on DNA ITS1, 5.8S, and ITS2 sequences. Subfamilies, tribes, and subtribes of Asteraceae are indicated. Numbers at the clade nodes are quartet puzzling values indicating percent consensus for membership in the clade. The outgroup designation is based on Susanna et al. (2006) who subdivided this group into *Arctoid*, *Cousinoid*, and *Saussurea* groups intermediate to Centaureinae and Carduinae.

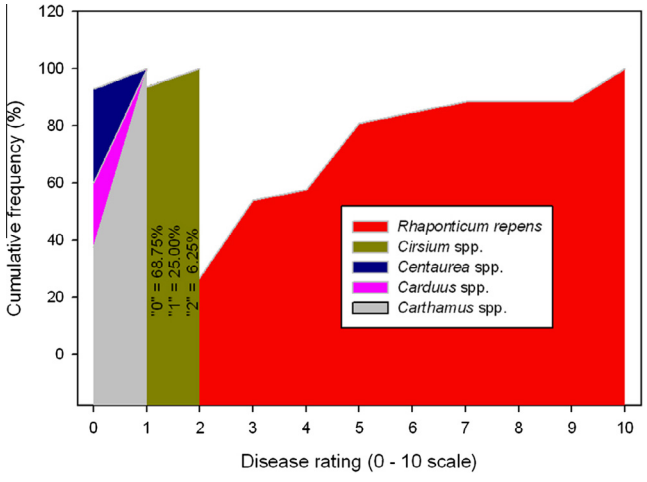

Fig. 4. Cumulative frequencies of disease ratings for *B. exigua* var. *rhapontica* on the target weed Russian knapweed (*Rhaponticum repens*) and non-target genera most closely related to the target weed. Disease ratings on *Cirsium* spp.: 68.75% of the ratings were "0", 25% were "1", and 6.25% were "2". Only three *Cirsium* spp. plants were rated "2".

Conidia on OA hyaline, smooth, oblong with obtuse ends, aseptate, straight, sometimes guttulate, 3.6–7.1 × 1.7–3.9 μm (average 5.2 × 2.7 μm). Conidial mass opaque with light purple tint.

Specimen examined: Turkey, from *R. repens* (*Asteraceae*), 2002, D. Berner, holotype BPI 843350, culture ex-type FDWSRU 02-059 = CBS 113651.

3.2. Host-range determination

3.2.1. Genetic relatedness among species

The genetic relatedness among the species evaluated is presented in a cladogram derived from maximum likelihood branch lengths from quartet puzzling analysis (Fig. 3). The cladogram was based on genetic distance matrix information generated from plant DNA ITS sequences. The relationships in this figure are supported by published trees (Hidalgo et al., 2006; Susanna et al., 2006) that indicate the unique and separate sub-clade of *Rhaponticum* and the closely related genera *Leuzea*, *Myopordon*, and *Oligochaeta* (none of which are present in N. America (Kartesz, 2014) among other Centaureinae. This sub-clade, termed the Stemmacantha group (Fig. 3) is clearly distinct from other genera of Centaureinae (Fig. 3). From this figure it is evident that species of *Centaurea* (including *Plectocephalus*, *Crupina* and *Serratula*) and *Carthamus* are most closely related to *R. repens* and other members of the *Rhaponticum* or Stemmacantha group (Fig. 3). Species of *Cirsium* and *Carduus* (Carduinae) were more distantly related. All other species were very distantly related in a highly divergent clade (Fig. 3).

3.2.2. Disease assessment

Cumulative frequencies of disease severity ratings for the most closely related non-target *Carduus* spp., *Carthamus* spp., *Centaurea*

spp., and *Cirsium* spp. along with frequencies of severity ratings for the target, *R. repens*, are shown in Fig. 4. The most severe disease rating was a "2" or estimated maximum of 20% of tissue diseased for species of *Cirsium*. However, the frequency for this rating was only 6.25%, while 93.75% of the ratings for species of this genus were "1" or less. The "2" rating was for three out of 21 plants of *Cirsium occidentale* var. *venustum*; ratings for the other 18 plants were "0". All three plants with ratings of "2" occurred in the same repetition of the tests; another three plants in the same repetition had ratings of "0". The overall ratings for species of *Cisium* were lower than those observed for all of the non-target species tested. Considering the comparatively remote genetic relatedness of *Cirsium* (subtribe Carduinae) to *Rhaponticum* (subtribe Centaureinae) (Fig. 3), only minor disease severity ratings of species of *Cirsium* would be expected with a host-specific pathogen like *B. ex. rhapontica*. More than 80.7% of the disease ratings for *R. repens* were above "1" and more than 73% were above "2". Results from mixed model analyses incorporating genetic relatedness with disease evaluation and damage data sharply illustrate differences in susceptibility among individual species and genera.

3.2.3. Analyses of disease and damage among species

Best Linear Unbiased Predictors (BLUPs) of disease severity rankings and logit values of disease incidence were generated for all 66 species (Table 2). BLUPs of logit values of disease incidence were significantly different from zero only for the three *Rhaponticum* spp. BLUPs of disease severity rankings were significantly different from zero, i.e., the BLUP indicated susceptibility, only for *R. repens*, *R. carthamoides*, *R. uniflorum*, and *L. berardioides*. *Rhaponticum uniflorum* and *L. berardioides* were not directly evaluated for disease reaction, but genetic distance information for these two was incorporated into the mixed model. BLUPs for disease severity ranking and logit values of disease incidence of all other species was statistically zero, i.e., not susceptible. Estimates of disease reactions for different genera (Table 3) showed that the order of susceptibility, based on disease incidence and severity was (most to least susceptible): *R. repens* > all other *Rhaponticum* spp. and *Leuzea berardoides* > *Carthamus* spp. > *Centaurea* and *Saussurea* spp. > *Carduus* spp. > *Cirsium* spp. Predicted disease incidence and severity for the *Cirsium* genus were not significantly different than zero and were the lowest of all closely related species (Table 3).

Best linear unbiased predictors, standard errors, and probabilities of greater *t*-values for differences in above-ground dry weights, between control and inoculated plants of a subset of the species evaluated for disease incidence and severity, are presented in Table 4. Overall, the differences between control and inoculated plants were not significant.

4. Discussion

Since identification of species of *Boeremia* and varieties of *B. exigua* is mainly based on host association (Aveskamp et al., 2009), the varietal epithet of *B. ex. rhapontica* seems appropriate. It has been found only on *R. repens* and was unique among the species of *Boeremia* and varieties of *B. exigua* in molecular analysis (Fig. 2). Only *B. ex. coffeae* was present within the same sub-clade

Table 2
Best linear unbiased predictors (BLUPs) for logit values, with back-transformed proportions of disease incidence, and for ranks of disease severity for disease caused by *Boeremia exigua* var. *rhapontica* on plant species related to the target weed *Rhaponticum repens*.

Genus species[a]	Disease incidence				Disease severity (0–10 scale)		
	Logit values[b]			Predicted proportion symptomatic plants	Ranks[c]		
	BLUP	Std error[d]	Pr > |*t*|[e]	Back-transformed proportion from BLUP values[f]	BLUP	Std error	Pr > |*t*|
Rhaponticum repens	4.68	1.54	0.0029	0.66	45.44	14.62	0.0023
Rhaponticum carthamoides	3.27	1.56	0.0386	0.32	31.95	14.84	0.0332
Rhaponticum uniflorum	3.09	1.57	0.0516	0.28	30.49	14.93	0.0433
Leuzea berardioides	2.90	1.55	0.0646	0.24	29.26	14.79	0.0501
Carthamus palaestinus	2.51	1.53	0.1045	0.18	21.81	14.55	0.1366
Carthamus oxyacantha	2.50	1.53	0.1046	0.18	21.83	14.50	0.1347
Carthamus tinctorius	2.50	1.53	0.1050	0.18	21.81	14.52	0.1357
Carthamus lanatus	2.43	1.55	0.1199	0.17	22.81	14.72	0.1240
Crupina vulgaris	2.31	1.55	0.1386	0.15	18.66	14.70	0.2066
Carthamus glaucus	2.28	1.54	0.1428	0.14	21.41	14.64	0.1461
Serratula coronata	2.16	1.57	0.1722	0.13	22.16	14.96	0.1410
Carthamus tenuis	2.01	1.60	0.2100	0.11	19.05	15.15	0.2110
Plectocephalus americanus	1.87	1.57	0.2346	0.10	18.54	14.87	0.2149
Plectocephalus rothrockii	1.79	1.57	0.2567	0.09	17.81	14.94	0.2353
Centaurea solstitialis	1.79	1.57	0.2567	0.09	14.90	14.89	0.3189
Centaurea montana	1.67	1.56	0.2867	0.08	17.69	14.88	0.2369
Centaurea x *moncktonii*	1.63	1.56	0.2984	0.08	16.87	14.82	0.2571
Centaurea melitensis	1.63	1.57	0.3036	0.08	16.98	14.95	0.2584
Carduus pycnocephalus	1.52	1.56	0.3312	0.07	14.11	14.81	0.3425
Cirsium vulgare	1.50	1.56	0.3382	0.07	14.93	14.81	0.3153
Carduus tenuiflorus	1.48	1.56	0.3456	0.07	14.45	14.85	0.3327
Centaurea sulphurea	1.46	1.57	0.3521	0.07	15.23	14.89	0.3085
Cirsium arvense	1.44	1.58	0.3629	0.07	14.03	14.98	0.3509
Saussurea candicans	1.41	1.59	0.3784	0.06	16.16	15.23	0.2906
Saussurea alpina	1.40	1.60	0.3829	0.06	15.87	15.29	0.3013
Centaurea diffusa	1.40	1.55	0.3699	0.06	14.57	14.69	0.3233
Saussurea nuda	1.36	1.60	0.3957	0.06	14.89	15.25	0.3306
Saussurea americana	1.34	1.59	0.4015	0.06	16.02	15.27	0.2962
Centaurea stoebe subsp. *micranthos*	1.34	1.56	0.3895	0.06	14.16	14.80	0.3403
Centaurea cyanus	1.31	1.58	0.4096	0.06	13.43	14.98	0.3716
Centaurea napifolia	1.29	1.58	0.4157	0.06	13.00	14.98	0.3869
Carduus nutans	1.27	1.57	0.4186	0.06	12.68	14.89	0.3962

Table 2 (*continued*)

Genus species[a]	Disease incidence				Disease severity (0–10 scale)		
	Logit values[b]			Predicted proportion symptomatic plants	Ranks[c]		
	BLUP	Std error[d]	Pr > |t|[e]	Back-transformed proportion from BLUP values[f]	BLUP	Std error	Pr > |t|
Silybum marianum	1.21	1.57	0.4404	0.05	12.28	14.91	0.4118
Cirsium scariosum var. *citrinum*	1.18	1.56	0.4506	0.05	12.54	14.87	0.4007
Centaurea calcitrapa	1.17	1.58	0.4630	0.05	12.18	15.00	0.4180
Cirsium fontinale var. obispoense	1.14	1.56	0.4647	0.05	12.37	14.83	0.4058
Cynara scolymus	1.11	1.58	0.4803	0.05	11.35	14.97	0.4494
Cirsium occidentale var. venustum	1.09	1.55	0.4811	0.05	11.98	14.75	0.4181
Cirsium rhothophilum	1.08	1.56	0.4884	0.05	11.96	14.80	0.4205
Cirsium hydrophilum var. hydrophilum	1.08	1.55	0.4882	0.05	11.97	14.80	0.4200
Cirsium fontinale var. fontinale	1.04	1.56	0.5074	0.04	11.39	14.84	0.4443
Arctium minus	0.99	1.61	0.5364	0.04	9.99	15.25	0.5139
Cirsium pitcheri	0.94	1.56	0.5480	0.04	10.48	14.87	0.4822
Picnomon acarna	0.93	1.62	0.5650	0.04	10.62	15.43	0.4925
Lactuca sativa	0.91	1.66	0.5839	0.04	5.54	15.74	0.7252
Onopordum acanthium	0.84	1.60	0.5982	0.04	9.56	15.20	0.5304
Taraxacum officinale	0.25	1.66	0.8771	0.02	1.00	15.77	0.9492
Liatris spicata	0.22	1.63	0.8925	0.02	2.99	15.43	0.8463
Achillea millefolium	0.08	1.71	0.9645	0.02	0.88	16.16	0.9565
Erigeron annuus	0.04	1.61	0.9776	0.01	2.43	15.35	0.8740
Baccharoides anthelmintica	0.03	1.63	0.9822	0.01	0.48	15.51	0.9756
Stokesia laevis	0.02	1.62	0.9878	0.01	2.58	15.40	0.8670
Hieracium albiflorum	−0.03	1.66	0.9821	0.01	−0.11	15.80	0.9941
Erigeron rhizomatus	−0.10	1.61	0.9483	0.01	0.55	15.47	0.9716
Erigeron clokeyi	−0.23	1.58	0.8818	0.01	−0.96	15.10	0.9492
Cichorium intybus	−0.24	1.64	0.8845	0.01	−1.49	15.65	0.9241
Krigia montana	−0.29	1.67	0.8594	0.01	−2.10	16.07	0.8960
Vernonia noveboracensis	−0.29	1.61	0.8531	0.01	−1.12	15.25	0.9415
Callistephus chinensis	−0.31	1.63	0.8490	0.01	−1.83	15.55	0.9066
Helianthus schweinitzii	−0.32	1.62	0.8401	0.01	0.52	15.43	0.9732
Vernonia missouriensis	−0.33	1.61	0.8345	0.01	−1.28	15.28	0.9331
Helianthus annuus	−0.35	1.62	0.8284	0.01	0.32	15.47	0.9831
Erigeron eatonii	−0.36	1.58	0.8189	0.01	−1.28	15.11	0.9324
Helianthus eggertii	−0.38	1.62	0.8126	0.01	0.06	15.44	0.9969
Linum usitatissimum	−0.39	1.65	0.8109	0.01	−1.92	15.69	0.9026
Solidago shortii	−0.40	1.64	0.8043	0.01	−1.68	15.78	0.9151

[a] Species are ordered by decreasing BLUP values for logits.
[b] Binomial disease incidence, number of diseased plants out of total number of inoculated plants for each repetition, converted to linear logit values as: $\ln(p/(1-p))$ where p = proportion diseased plants. Average logit values for each species in each repetition were analyzed.
[c] Ranks of disease severity ratings. Ratings for each inoculated plant were based on a 0–10 scale where "0" = no disease and "10" = 100% diseased plant tissue. Ranks of ratings were computed across all plants in all repetitions and the average ranks for each species in each repetition were analyzed.
[d] Standard error of the prediction based on BLUP of random species effect without intercept.
[e] Pr > |t| based on BLUP of random species effect without intercept.
[f] Predicted proportions of symptomatic plants obtained by back-transformation: exp(BLUP of logit value + intercept)/(1 + exp(BLUP of logit value + intercept)); intercept = −4.0175.

Table 3
Estimates of Best linear unbiased predictors (BLUPs) of logit values of disease incidence and of ranks of disease severity for disease caused by *Boeremia exigua* var. *rhapontica* on plant genera related to the target weed, *Rhaponticum repens*, and of differences in incidence and severity between *R. repens* and other genera.

	Disease incidence			Rank of disease severity (0–10 scale)		
	BLUP	Std error	Pr > |t|	BLUP	Std error	Pr > |t|
Estimates						
All other *Rhaponticum* and *Leuzea* spp.	3.09	1.55	0.0505	30.57	14.71	0.0426
All *Carthamus* spp.	2.38	1.53	0.1254	21.45	14.49	0.1447
All *Centaurea* spp.	1.38	1.39	0.3244	14.18	13.17	0.2865
All *Saussurea* spp.	1.38	1.58	0.3844	15.74	15.05	0.3004
All *Carduus* spp.	1.43	1.55	0.3605	13.75	14.70	0.3539
All *Cirsium* spp.	1.17	1.54	0.4508	12.41	14.63	0.4002
R. repens minus all other *Rhaponticum* spp. and *Leuzea berardioides*	1.58	0.37	<.0001	14.86	3.58	.0001
R. repens minus all *Carthamus* spp.	2.30	0.50	<.0001	23.98	4.77	<.0001
R. repens minus all *Centaurea* spp.	3.29	0.47	<.0001	31.25	4.52	<.0001
R. repens minus all *Saussurea* spp.	3.29	0.67	<.0001	29.69	6.65	<.0001
R. repens minus all *Carduus* spp.	3.25	0.65	<.0001	31.69	6.25	<.0001
R. repens minus all *Cirsium* spp.	3.51	0.57	<.0001	33.02	5.54	<.0001

as *B. ex. rhapontica*. The variety *B. ex. coffeae* has only *Coffea arabica* as its host, and this plant species is not closely related to *R. repens*.

From Fig. 2 it is evident that some *B. exigua* varieties and *Boeremia* spp. are host specific while others seem to have a very broad host range. Original epithets of *Boeremia* (and *Phoma*) species and varieties were based on the hosts from which they were collected and, later, speciation was based on characteristics of the fungi on artificial culture media (Boerema et al., 2004). Molecular genetics

Table 4
Best linear unbiased predictors (BLUPs) for differences in above-ground dry weights, between mean non-inoculated controls and individual inoculated plants for disease caused by *Boeremia exigua* var. *rhapontica*.[a]

Genus species	BLUP	Std error	$P > \|t\|$
Rhaponticum repens	0.2411	0.2177	0.2757
Rhaponticum carthamoides	0.1945	0.2179	0.3780
Rhaponticum uniflorum	0.1884	0.2181	0.3935
Carthamus oxyacantha	0.1698	0.2178	0.4409
Carthamus tinctorius	0.1678	0.2178	0.4462
Serratula coronata	0.1522	0.2183	0.4904
Centaurea montana	0.1429	0.2183	0.5170
Cynara scolymus	0.1403	0.2180	0.5241
Cirsium vulgare	0.1402	0.2179	0.5241
Saussurea alpina	0.1397	0.2186	0.5272
Cirsium occidentale	0.1380	0.2179	0.5305
Plectocephalus rothrockii	0.1360	0.2178	0.5364
Cirsium pitcheri	0.1357	0.2179	0.5376
Plectocephalus americanus	0.1337	0.2180	0.5435
Picnomon acarna	0.1307	0.2187	0.5540
Cirsium fontinale var. *fontinale*	0.1298	0.2179	0.5552
Crupina vulgaris	0.1298	0.2182	0.5556
Centaurea x *moncktonii*	0.1265	0.2178	0.5653
Centaurea sulphurea	0.1246	0.2179	0.5711
Erigeron rhizomatus	0.1242	0.2198	0.5755
Centaurea solstitialis	0.1240	0.2178	0.5729
Centaurea napifolia	0.1239	0.2180	0.5734
Carduus nutans	0.1237	0.2180	0.5743
Centaurea melitensis	0.1234	0.2178	0.5746
Centaurea diffusa	0.1225	0.2178	0.5774
Centaurea cyanus	0.1213	0.2181	0.5814
Carduus tenuiflorus	0.1213	0.2180	0.5816
Solidago shortii	0.1206	0.2193	0.5861
Centaurea calcitrapa	0.1205	0.2179	0.5838
Centaurea stoebe subsp. micranthos	0.1203	0.2178	0.5843
Callistephus chinensis	0.1138	0.2185	0.6057
Helianthus schweinitzii	0.1087	0.2202	0.6247
Helianthus eggertii	0.1073	0.2202	0.6292
Cichorium intybus	0.1023	0.2201	0.6450
Krigia montana	0.0902	0.2201	0.6844
Estimate	BLUP	Std error	$P > \|t\|$
Estimates of differences in weights between non-inoculated control plants minus inoculated plant weights			
Rhaponticum repens	0.2411	0.2177	0.2748
All other spp.	0.1283	0.1976	0.5198
Rhaponticum repens minus all other species	0.1091	0.0761	0.1596

[a] Differences in above-ground oven-dry weights calculated by subtracting the dry weight of each inoculated plant of each species in each repetition from the mean dry weight of all non-inoculated control plants of the same species in the same repetition.

techniques have only recently been employed to separate, and often rename, these fungi (Aveskamp et al., 2009). Thus, nomenclature of this complicated group of fungi is evolving.

Results of analyses of both disease incidence and severity indicate that *B. ex. rhapontica* from *R. repens* has a very narrow host range that is limited to species in the *Rhaponticum* group (Hidalgo et al., 2006) of Centaureinae. Very good levels of disease were recorded on the target species, *R. repens*, and no species, other than non-native *Rhaponticum* or *Leuzea*, had significantly non-zero predictions of disease incidence or severity, i.e., non-significant probability that these species were susceptible to *B. ex. rhapontica*. Since conditions in nature are much less favorable for disease, than the optimum conditions for disease in our greenhouse tests, and spore concentrations in nature would likely be much lower than those used in these artificial inoculations, no closely related non-target species, including *Cirsium* species, would likely be attacked or damaged by *B. ex. rhapontica* in nature. In terms of anticipated susceptibility to *B. ex. rhapontica*, based on genetic inter-relatedness (the presumed basis for centrifugal phylogenetic testing method, Wapshere, 1974) the most likely order of susceptibility would be: *Rhaponticum* > *Centaurea* (including *Plectocephalus*, *Crupina* and *Serratula*) & *Carthamus* > *Saussurea* > *Cirsium* & *Carduus* > all other species. All host range determination results were obtained in greenhouse tests with optimum conditions for disease development. Consequently, some plants in these tests developed a few and/or minor lesions attributable to *B. ex. rhapontica*. This is typical of facultative saprophytes, like *B. ex. rhapontica*, that can colonize plant tissue previously damaged by other means and give rise to apparent disease lesions under optimum conditions. This phenomenon, termed "induced susceptibility" under greenhouse conditions, is presented in Bruckart et al. (1985) and Evans (2000). However, "induced susceptibility" is misleading since the presence of a few and/or minor lesions does not necessarily indicate disease or susceptibility but might indicate induction of plant defense mechanisms, i.e., resistance. There are numerous types of host defense responses to challenges by plant pathogens, and overviews of some of these responses are presented by Heath (1982, 1991, 2000). Disease severity ratings of "2" or less on some plants of species that had neither significant disease incidence nor severity e.g., *Cirsium occidentale* var. *venustum* and other *Cirsium* spp. might be indicative of resistant responses. In addition to defense responses, saprophytic activity by the parasite also confounds absolute objective evaluations of susceptibility, and leave probabilities of susceptibility (significant disease incidence or severity) as the only scientific alternative in host-range determination. Incorporation of genetic relationships among species into host evaluation tests, allows probabilities of susceptibility to be broadly-applicable to species rather than just material tested in a greenhouse.

The lack of significant differences among species in above-ground dry weight differences between control and inoculated plants (Table 4) was due to the large amount of variability in the differences in above-ground dry weights, as reflected by the standard errors. The large amount of variability was likely due to variable plant sizes among relatively small plants, growth differences among plant species, and possible differential disease and damage reaction among these species. Thus, although differences in above-ground dry weights in Table 4 were not significantly different than zero, the difference (above-ground damage) for *R. repens* was nearly twice that for any other species, except *Rhaponticum* species (Table 4).

As with other creeping perennials, the key to controlling established *R. repens* patches is to stress the weed and cause it to expend root reserves (Beck, 1998). Field tests on control of *R. repens* with *B. ex. rhapontica* should be conducted to test whether the fungus can reduce root reserves of *R. repens* in the field as indicated by greenhouse results. As the results of this study indicate that *B. ex. rhapontica* would not pose any risk to plants of value in North America, field studies on control effectiveness should be done to determine whether release of *B. ex. rhapontica* in N. America would be beneficial to controlling this invasive and problematic weed.

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