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

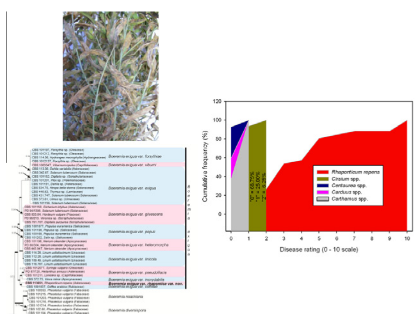
Dana Berner[a,*], Craig Cavin[a], Joyce H.C. Woudenberg[b], Berna Tunali[c], Orhan Büyük[d], Bayram Kansu[c]

[a] USDA – ARS – Foreign Disease-Weed Science Research Unit, 1301 Ditto Avenue, Ft. Detrick, MD 21702-5023, USA
[b] CBS – KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
[c] Ondokuz Mayis University, Agricultural Faculty, Department of Plant Protection, 55139 Atakum, Samsun, Turkey
[d] Plant Protection Central Research Institute, 06172 Yenimahalle, Ankara, Turkey

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*

* Corresponding author.
E-mail address: dana.berner@ars.usda.gov (D. Berner).

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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.