Available online at www.sciencedirect.com
SCIENCE DIRECT
Applied Soil Ecology 32 (2006) 27–37
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Soilborne microorganisms of *Euphorbia* are potential biological control agents of the invasive weed leafy spurge

Robert J. Kremer[a,*], Anthony J. Caesar[b], Thouraya Souissi[c]

[a] *USDA, Agricultural Research Service, Cropping Systems & Water Quality Research Unit and Department of Soil, Environment & Atmospheric Sciences, University of Missouri, 302 Natural Resources Building, Columbia, MO 65211, USA*
[b] *USDA, Agricultural Research Service, Northern Plains Agricultural Research Center, 1500 N. Central Ave., Sydney, MT 59270, USA*
[c] *Institut National Agronomique de Tunisie, Tunis, Tunisia*

Received 13 January 2004; received in revised form 22 July 2004; accepted 29 December 2004

Abstract

Leafy spurge (*Euphorbia esula-virgata*), a native of Eurasia, is a serious invasive weed of grasslands of the northern Great Plains of the U.S. and prairie provinces of Canada. Leafy spurge is very difficult to control with herbicides, insect biological control agents, and other cultural practices. Previous field investigations revealed pathogen–insect interactions on the roots of leafy spurge leading to mortality. In order to exploit this synergistic relationship as an effective biological control strategy, we undertook an exploration of Europe for soilborne fungi and rhizosphere bacteria on *Euphorbia* spp. growing in a wide variety of soils in different landscapes. All microbial cultures were screened for growth suppressive or disease potential on leafy spurge plants or callus tissue. Study objectives were to determine relationships of some edaphic factors and host plant conditions with biological control activity, and to screen rhizobacteria isolated from *Euphorbia* spp. for traits that might contribute to suppression of leafy spurge growth. The most virulent soilborne fungal strains of *Fusarium* and *Rhizoctonia* species, based on greenhouse pathogenicity tests, were isolated from roots of *Euphorbia* spp. with insect feeding damage. High proportions (>50%) of rhizobacteria were classified as deleterious rhizobacteria (DRB) using a callus tissue bioassay. *Euphorbia* spp. at sites with high DRB numbers displayed severe fungal disease symptoms and supported insect infestations. Selected soil properties were not correlated with potential biocontrol activity of microbes on leafy spurge; however, insect presence and disease ratings were associated with incidence of growth-suppressive microbes. Certain physiological traits (i.e., exopolysaccharides and hydrogen cyanide production) were good indicators of deleterious activity of rhizobacteria. Our study illustrates that the most effective condition for inducing disease and subsequent mortality of leafy spurge includes a synergism between plant-associated microorganisms and root-damaging insects. Furthermore, the results are valuable for identifying sites for collecting soilborne microorganisms on weeds in their native range for evaluation as biocontrol agents in their invasive range.
Published by Elsevier B.V.

Keywords: *Aphthona* spp.; Deleterious rhizobacteria; *Fusarium* spp.; Rhizosphere ecology; *Rhizoctonia* spp.; Root-feeding insects; Soilborne fungal pathogens; Synergism

1. Introduction

Leafy spurge (*Euphorbia esula-virgata* L.) is an invasive, deep-rooted perennial weed that reproduces both by seed production and by vegetative propagation from apical buds located on the underground portion of the stem (crown) and lateral roots. Anderson et al. (2000) reported that leafy spurge infests approximately 2 million ha of land in the northern Great Plains of the U.S. and the prairie provinces of Canada. Leafy spurge is a highly successful competitor for resources and space, a result of its adaptation to a broad range of environmental conditions and its aggressive, extensive

* Corresponding author. Tel.: +1 573 882 6408; fax: +1 573 884 5070.
E-mail address: KremerR@missouri.edu (R.J. Kremer).

0929-1393/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.apsoil.2004.12.009

root system, the primary characteristics leading to its invasive nature. Although relationships between soil microorganisms and invasive plant species have received little attention, large changes in landscape vegetation suggest simultaneous alteration in the structure and activity of the soil microbial community (Klironomos, 2002). Vegetation composition changes under dense leafy spurge infestations may affect microbial community functioning by providing large amounts of organic residues different from native plant composition, and by altering the rhizosphere environment. Soil microbial inhabitants that benefit the proliferation of the invasive plant community may also be selected. Growth of desirable plant species is likely reduced by competition although leafy spurge may also limit neighboring plant growth through allelopathy (Lajeunesse et al., 1999). Beef production is indirectly decreased through impaired grazing of adjacent forage caused by latex-based chemicals present in the leafy spurge plant, which is toxic to cattle (Lajeunesse et al., 1999). Because leafy spurge is a major threat to vegetation in pastures, rangelands, and native habitats, it is listed as 1 of the 12 least desirable invasive species in U.S. ecosystems (Stein and Flack, 1996).

The vegetative reproduction trait of leafy spurge resists many weed control tactics because crown or root buds can regenerate new plants after foliage is treated with herbicides, mowing, biological control agents, burning, or grazing. Long-term control with herbicides is difficult to achieve (Markle and Lym, 2001); thus, it is imperative to develop effective biological control for use in leafy spurge management programs. Leafy spurge is considered an ideal target for biological control because the habitats it invades are generally incompatible with herbicide control and its perennial nature can provide a consistent food source for biological control organisms (Kirby et al., 2000).

Biological control of leafy spurge has centered on introduction and release of a suite of insect natural enemies. Insects for biological control have been comprised mainly of flea beetles (*Aphthona* spp.), the larvae of which feed specifically on leafy spurge roots. Examination of the impact of flea beetles released for control of leafy spurge in North America indicated successful insect establishment at numerous sites but with little or no measurable impact on the weed stand (Caesar, 2000). However, successful biological control observed as rapid declines in leafy spurge stands at release sites most frequently coincided with the presence of soilborne pathogens associated with root-feeding *Aphthona* larvae (Caesar, 2000). These observations strongly suggested that soilborne plant pathogens including *Rhizoctonia* and *Fusarium* associated with damage caused by *Aphthona* and other insects should be collected to supplement insect releases for more rapid and consistent suppression of leafy spurge infestations.

Biological control strategies based on the use of deleterious rhizobacteria (DRB) take advantage of selected, non-parasitic bacteria that colonize plant roots and suppress plant growth (Kremer et al., 1990; Kremer and Kennedy, 1996; Nehl et al., 1997). The biological control potential of DRB toward leafy spurge has been demonstrated using suspended cell and callus tissue culture bioassays (Kremer et al., 1998; Souissi and Kremer, 1994, 1998). DRB applied to soil in leafy spurge-infested field plots in South Dakota suppressed growth to a limited extent by decreasing root weight and root carbohydrate content (Brinkman et al., 1999).

Mechanisms by which rhizobacteria suppress plant growth are not fully understood but may include overproduction of indoleacetic acid (Sarwar and Kremer, 1995; Xie et al., 1996), production of siderophores (Loper and Buyer, 1991), extracellular polysaccharides (Fett et al., 1989; Kelman, 1954), and hydrogen cyanide (Kremer and Souissi, 2001; Owen and Zdor, 2001; Schippers et al., 1987). The presence of any or all of these traits may indicate potential growth-suppressive activity and may be useful in selecting DRB from rhizosphere bacterial isolates.

More information is needed on how soilborne fungi and rhizosphere bacteria interact with environmental conditions (soil properties, climate, etc.) to successfully colonize the rhizosphere and roots and express deleterious traits so that biological control efficacy is more predictable (Horwath et al., 1998). Little is known about the influence of edaphic factors on leafy spurge biocontrol activity by soil microorganisms. Because the synergism between insects and pathogens has been largely overlooked, a survey was conducted for soilborne pathogenic fungi and DRB associated with damage caused by root-feeding insects attacking *Euphorbia* spp. in its native range in Europe. Collection of organisms with biological control potential from various sites in the native range is a necessary step in the development of biological management of invasive weed species (Harley and Forno, 1992). Our objectives were to determine relationships of selected edaphic factors and host plant conditions at collection sites with biological control activity of soilborne microorganisms, and to screen rhizobacteria from *Euphorbia* rhizospheres for putative DRB traits that might contribute to suppression of leafy spurge growth.