BIOLOGICAL CONTROL 4, 373–381 (1994)

Evaluation of Endophytic Bacteria as Potential Biological Control Agents for Oak Wilt

DAVID S. BROOKS,* CARLOS F. GONZALEZ,*,[1] DAVID N. APPEL,* AND TED H. FILER†

*Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843; and †USDA Southern Forest Experiment Station, Stoneville, Mississippi 38776

Received October 12, 1993; accepted May 3, 1994

Endophytic bacteria were isolated from surviving live oaks (*Quercus fusiformis*) in Texas, where oak wilt is epidemic, and evaluated as potential biological control agents for the disease. Of the 889 bacterial isolates tested, 183 showed *in vitro* inhibition of the pathogen, *Ceratocystis fagacearum*. Six isolates were further evaluated for colonization of containerized Spanish oaks (*Quercus texana*) and live oaks. In general, when injected into the stems, *Bacillus* species colonized Spanish oaks more extensively than *Pseudomonas* species; whereas, the opposite was true for live oaks. In containerized live oaks inoculated with the oak wilt pathogen, preinoculation with *Pseudomonas denitrificans* 1-15 reduced the number of diseased trees by 50% and in the same trial decreased the percentage of crown loss by 17%. In a second trial, no reduction in numbers of diseased trees was observed; however, preinoculation with either strain 1-15 or *Pseudomonas putida* 5-48 significantly reduced crown loss. These studies indicate that a potential exists for the utilization of selected bacteria to control oak wilt.

KEY WORDS: ***Ceratocystis fagacearum; Quercus fusiformis; Quercus virginiana; Quercus texana; Pseudomonas denitrificans; Pseudomonas putida; Erwinia herbicola; Bacillus pumilis; Bacillus alvei;* biocontrol.**

INTRODUCTION

Oak wilt is a vascular disease of oaks caused by the fungus *Ceratocystis fagacearum* (Brentz) Hunt. In central Texas, an oak wilt epidemic is currently devastating live oaks (*Quercus virginiana* Mill.) in urban and rural areas (Appel and Maggio, 1984). The wide distribution of live oaks and their significant aesthetic and monetary value have led to intense public pressure for developing reliable inexpensive methods to control the disease. Traditionally, oak wilt has been controlled by breaking root connections between diseased and healthy trees (Gibbs and French, 1983; MacDonald and Hindal, 1981). Removal of diseased Spanish oaks (*Quercus texana* Small) prevents the formation of fungal mats, the only known source of inoculum for long-distance transmission of the pathogen by nitidulids or sap beetles (Gibbs and French, 1983).

The current practice to prevent root transmission of the pathogen is to trench around diseased oaks and remove susceptible trees within 30 m. However, trenching is an expensive treatment and is difficult in the shallow rocky soils typical of central Texas. Also, the large size of many rural oak wilt centers makes trenching around them impractical. In urban areas, trenching may damage buried water and utility lines. Eradication of trees is also unpopular with property owners since oak trees provide valuable shade, and successful control requires the removal of symptomless trees.

Recent research has shown that intravascular injection with the fungicide propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) has promise for oak wilt control (Appel and Kurdyla, 1992). However, concerns exist over the use of fungicides because of their persistence in the environment. Also, there is potential for development of a fungicide-resistant pathogen population. These concerns warrant the investigation of alternative strategies such as biological control.

Biological control has been studied in other vascular wilt diseases of trees such as Dutch elm disease (DED) (Myers and Strobel, 1983; O'Brien *et al.*, 1984; Scheffer, 1983; Scheffer *et al.*, 1989a,b; Shi and Brasier, 1986) and verticillium wilt of maples (Hall *et al.*, 1986). Microorganisms showing *in vitro* inhibition of both the DED and verticillium wilt pathogens include *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp., and *Streptomyces* spp. (Gregory *et al.*, 1984; Hall *et al.*, 1986; Murdoch *et al.*, 1984; Myers and Strobel, 1983). Several biological control studies of DED have focused on the use of these microorganisms as agents for control (Myers and Strobel, 1983; Scheffer, 1983; Scheffer *et al.*, 1989a,b; Shi and Brasier, 1986). However, attempts to control DED using

[1] To whom correspondence should be addressed.

1049-9644/94 $6.00