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Biological Control 36 (2006) 358–367
Biological Control
www.elsevier.com/locate/ybcon

Integrated biological control of bacterial speck and spot of tomato under field conditions using foliar biological control agents and plant growth-promoting rhizobacteria

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Received 27 June 2005; accepted 12 September 2005
Available online 21 October 2005

Abstract

Integration of foliar bacterial biological control agents and plant growth promoting rhizobacteria (PGPR) was investigated to determine whether biological control of bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato*, and bacterial spot of tomato, caused by *Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas vesicatoria*, could be improved. Three foliar biological control agents and two selected PGPR strains were employed in pairwise combinations. The foliar biological control agents had previously demonstrated moderate control of bacterial speck or bacterial spot when applied as foliar sprays. The PGPR strains were selected in this study based on their capacity to induce resistance against bacterial speck when applied as seed and soil treatments in the greenhouse. Field trials were conducted in Alabama, Florida, and California for evaluation of the efficacy in control of bacterial speck and in Alabama and Florida for control of bacterial spot. The foliar biological control agent *P. syringae* strain Cit7 was the most effective of the three foliar biological control agents, providing significant suppression of bacterial speck in all field trials and bacterial spot in two out of three field trials. When applied as a seed treatment and soil drench, PGPR strain *Pseudomonas fluorescens* 89B-61 significantly reduced foliar severity of bacterial speck in the field trial in California and in three of six disease ratings in the field trials in Alabama. PGPR strains 89B-61 and *Bacillus pumilus* SE34 both provided significant suppression of bacterial spot in the two field trials conducted in Alabama. Combined use of foliar biological control agent Cit7 and PGPR strain 89B-61 provided significant control of bacterial speck and spot of tomato in each trial. In one field trial, control was enhanced significantly with combined biological control agents compared to single agent inoculations. These results suggest that some PGPR strains may induce plant resistance under field conditions, providing effective suppression of bacterial speck and spot of tomato, and that there may be some benefit to the integration of rhizosphere-applied PGPR and foliar-applied biological control agents.
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Keywords: *Bacillus pumilus*; *Pseudomonas fluorescens*; *Pseudomonas putida*; *Pseudomonas syringae*; *Xanthomonas campestris* pv. *vesicatoria*; *Xanthomonas vesicatoria*; Bacterial speck; Bacterial spot; Biological control; Plant growth-promoting rhizobacteria; Induced systemic resistance; Tomato; *Lycopersicon esculentum*

1. Introduction

Bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato*, and bacterial spot of tomato, caused by *Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas vesicatoria*, are among the most economically important bacterial diseases in many tomato-growing regions of North America and the world (Goode and Sasser, 1980). Lesions occur on leaves and may cause an entire leaflet to turn yellow and drop. As the diseases progress, the lesions may spread to stems, petioles, and flowers. Yield reductions can result from the reduced photosynthetic capacity of infected foliage, leaf defoliation, flower abortion, and from

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1049-9644/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2005.09.003

lesions on the fruit that render them unsuitable for the fresh market or for processing. Bacterial speck is more severe under cool and humid conditions, whereas bacterial spot is favored by warm and rainy weather. Both diseases may cause significant reductions in tomato yield, especially if the infection appears early in the season (Pohronezny and Volin, 1983; Yunis et al., 1980). The efficacy of current strategies for control of bacterial speck and spot is limited. Cultural practices do not provide sufficient control of the diseases and have not been generally adopted by commercial growers (Conover and Gerhold, 1981; Lawton and MacNeill, 1986). Copper bactericides, applied alone or in combination with ethylenebis-dithiocarbamate (EBDC) fungicides, have been traditionally used to control the diseases (Conlin and McCarter, 1983; Conover and Gerhold, 1981; Jardine and Stephens, 1987; Jones and Jones, 1985; Marco and Stall, 1983). However, ineffective disease suppression due to development of copper resistance in the pathogen populations in many areas (Bender and Cooksey, 1986; Marco and Stall, 1983; Pernezny et al., 1995; Silva and Lopes, 1995) and increased public concern about detrimental effects of pesticide residues have made alternative or complementary methods to control these diseases desirable.

Considerable efforts have been directed to identify genetic resistance to bacterial speck and spot (Jones et al., 1998; Pitblado and MacNeill, 1983; Pitblado et al., 1984; Scott et al., 1997). The *Pto* gene has been demonstrated to confer resistance in tomato to race 0 strains of *P. syringae* pv. *tomato* that express the gene *avrPto* (Martin et al., 1993; Ronald et al., 1992). However, the occurrence of race 1 strains of the pathogen lacking the *avrPto* may hamper the mass release of *Pto* into commercial cultivars (Donner and Barker, 1996; Habazar and Rudolph, 1997; Lawton and MacNeill, 1986). With regard to bacterial spot of tomato, commercial cultivars resistant to the disease are not available, partially because the effectiveness of disease resistance does not appear to persist (Jones et al., 1998; Scott et al., 1997). Another control practice that may be applicable to tomato is chemically induced systemic acquired resistance (SAR). A synthetic compound, acibenzolar-*S*-methyl (Actigard; Bion) has been reported to induce SAR and provide significant suppression of bacterial speck and spot in field trials (Abbasi et al., 2002; Louws et al., 2001; Wilson et al., 2002). Thus, chemically induced SAR may be an effective method for control of bacterial speck and spot, although the application of these chemicals remains to be optimized since negative impact on plant growth or yield have been reported (Csinos et al., 2001; Louws et al., 2001; Romero et al., 2001).

Biological control may provide an additional tool to these chemical approaches for bacterial disease management. Bacterial biological control agents are now commercially available for the control of crown gall, fire blight of pear and several other diseases (Backman et al., 1997; Kloepper, 1993; Lindow et al., 1996; Lindow and Wilson, 1999; Wilson, 1997, 2004; Wilson and Backman, 1999). Selected bacteriophages have been demonstrated to be effective under greenhouse and field conditions for control of bacterial spot of tomato and have been commercialized (Balogh et al., 2003; Flaherty et al., 2000; Obradovic et al., 2004). Although less effort has been directed toward the use of nonpathogenic bacteria for control of bacterial speck and spot, recent studies showed that some biological control agents, especially a foliar bacterial strain *P. syringae* Cit7, consistently suppressed bacterial speck and spot under field conditions at several locations in North America (Byrne et al., 2005; Wilson et al., 2002). It was speculated that the bacterial strain Cit7 provided protection of tomato via mechanisms including induced resistance (Wilson et al., 2002). While induced plant resistance by foliar bacterial biological control agents has not been intensively studied, induced systemic resistance (ISR) by plant growth-promoting rhizobacteria (PGPR) has been the subject of many investigations in recent years (Kloepper et al., 1999, 1992; van Loon et al., 1998; Wei et al., 1996; Zehnder et al., 2001). Treatment of seed or root with PGPR significantly reduced severity of anthracnose, angular leaf spot and cucurbit wilt diseases on cucumber (Raupach and Kloepper, 1998; Raupach and Kloepper, 2000; Wei et al., 1996; Zehnder et al., 2001), and southern blight, bacterial wilt, *Tobacco mosaic virus* (TMV) and *Tomato mottle virus* (ToMoV) in tomato (Anith et al., 2004; Jetiyanon et al., 2003; Murphy et al., 2000; Zehnder et al., 2001). It was hypothesized, therefore, that some PGPR strains might provide systemic protection against bacterial speck and spot of tomato under natural environmental conditions.

The main goal of this study was to determine whether the control of bacterial speck and spot of tomato could be improved through the combined use of foliar biological control agents applied to the leaves and ISR-eliciting PGPR applied to the roots. Three foliar biological control agents were included in the study: *P. syringae* strain Cit7; *Pseudomonas fluorescens* strain A506; and *P. putida* strain B56 (Wilson et al., 2002). While these foliar bacterial strains have been shown to provide protection against both bacterial speck and bacterial spot of tomato, only a relatively moderate level of disease control was achieved (Byrne et al., 2005; Wilson et al., 2002). Hence, a collection of PGPR strains was screened for the capacity to elicit ISR in tomato plants and used to determine whether some combinations of PGPR and foliar biological control agents could improve disease control efficacy.

2. Materials and methods

2.1. Bacterial strains

Bacterial strains *P. fluorescens* A506 (Lindow et al., 1996) and *P. syringae* Cit7 (Lindow, 1985) were provided by S.E. Lindow (University of California, Berkeley, CA). *Pseudomonas putida* strain B56 was isolated from tomato leaves in Florida (Wilson et al., 2002). In a previous study, these bacterial strains significantly reduced foliar severity of bacterial speck of tomato (Wilson et al., 2002). *Pseudomonas syringae* Cit7 and *P. putida* B56 were also moderately

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