Biological Control 67 (2013) 361–372

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Efficacy of selected streptomycetes and a streptomycete + pseudomonad combination in the management of selected bacterial and fungal diseases of field tomatoes

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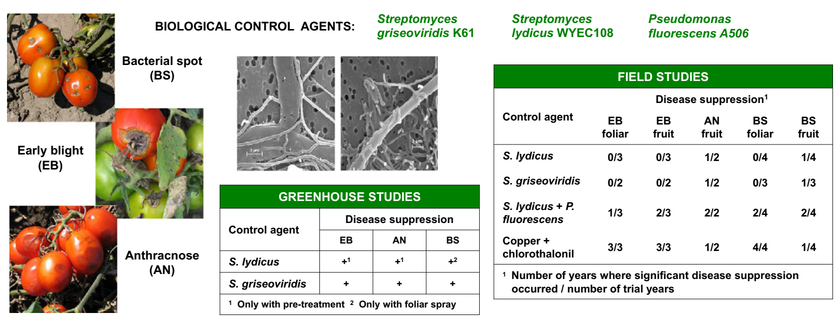
HIGHLIGHTS

- Disease control with *Streptomyces griseoviridis* (Sg) or *Streptomyces lydicus* (Sl).
- Stable populations (⩾10^3 CFU/g) of both maintained on foliage for two weeks or more.
- Early blight, anthracnose and bacterial spot suppressed in greenhouse but not field.
- A Sl + *Pseudomonas fluorescens* combination more effective than either microbe alone.
- This combination more effective than copper for anthracnose or spot on field fruit.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 June 2013
Accepted 8 September 2013
Available online 17 September 2013

Keywords:
Solanum lycopersicum
Bacterial spot
Early blight
Anthracnose
Streptomyces lydicus
Streptomyces griseoviridis

ABSTRACT

The aim of this study was to determine if *Streptomyces griseoviridis* K61 (Sg) or *Streptomyces lydicus* WYEC108 (Sl), known for the production of antimicrobial metabolites, would be of value in a biological control program focusing not only on fungal diseases early blight and anthracnose but also on bacterial spot of field tomatoes. Also included in the study was *Pseudomonas fluorescens* A506 (Pf), a biological control agent with known antibacterial properties. Growth of the fungi was suppressed by both streptomycetes with Sl showing the most activity. Scanning electron microscopy revealed that both streptomycetes established close contact with both pathogens but only Sg caused a turgor loss and hyphal degradation. Representative strains of the bacterial spot pathogen were strongly inhibited by Pf, moderately inhibited by Sg but unaffected by Sl. Both streptomycetes suppressed anthracnose on fruit and early blight on detached leaves; however, Sl provided protection only as a pretreatment. In three of six experiments, foliar sprays of Sl or soil drenches of Sg were equivalent to soil drenches of the plant activator acibenzolar-S-methyl in suppressing bacterial spot on tomato seedlings. Both streptomycetes maintained populations of ⩾10^3 CFU g^{-1} on tomato seedlings for two weeks minimum. In a four-year field study, neither streptomycete was as effective as the copper standard at controlling early blight or bacterial spot on foliage. However, the most effective treatment for anthracnose was a *S. lydicus* WYEC108 + *P. fluorescens* A506 treatment combination. This treatment combination also was the most effective at reducing bacterial spot severity on fruit.

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http://dx.doi.org/10.1016/j.biocontrol.2013.09.005

1. Introduction

Bacterial spot, caused by *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria*, *Xanthomonas perforans* or *Xanthomonas gardneri* (Jones et al., 2004), early blight, caused by Alternaria solani, and anthracnose, caused by *Colletotrichum coccodes*, have been serious and persistent disease problems for field tomato growers in several tomato (*Solanum lycopersicum* L.) growing regions of the US as well as Canada (Jones et al., 1991). None of the commercial tomato cultivars grown in Ontario have resistance (J. Dick, Tomato Solutions, personal communication) to *X. gardneri* or *X. perforans*, the predominant bacterial spot species found in Ontario (Cuppels et al., 2006). Likewise, the commercial tomato varieties currently used in Ontario do not have a high degree of resistance to either early blight or anthracnose (Kemmitt, 2002) (S. Loewen, Univ. of Guelph, personal communication). Thus, for polycyclic diseases such as these, control is based on the use of pathogen-free seed and transplants, crop rotation, field sanitation and the frequent application of chemical sprays such as copper hydroxide plus chlorothalonil or mancozeb. Although copper resistance is infrequent among Ontario isolates of the bacterial spot-causing xanthomonads (BSX), it has been reported for BSX strains from other tomato-growing regions of North America (Jones et al., 1991, Ritchie and Dittapongpitch, 1991). Because of concerns over the effectiveness of chemical pesticides and their impact on human health and the environment (Flemming and Trevors, 1989; Dumestre et al., 1999), an integrated, multifaceted, more biologically-based disease management strategy is warranted.

A number of control practices that do not rely on copper have been tested for the ability to suppress bacterial spot of tomato. The compound acibenzolar-S-methyl (ASM; Actigard™ 50WG or Bion® 50WG, Syngenta, Basel Switzerland), a functional analog of salicylic acid, mimics the natural systemic activated resistance (SAR) response found in many plants to a broad spectrum of plant pathogens (Oostendorp et al., 2001). It has field efficacy equivalent or superior to copper-mancozeb or copper-chlorothalonil standards (Louws et al. 2001; Abbasi et al., 2002; Roberts et al., 2008; Huang et al., 2012). Unfortunately, application of this compound at the recommended rate may have a negative impact on marketable yield, depending upon environmental conditions and the stress level of the plants. Although recently registered in Canada (Actigard™ 50WG, Syngenta Crop Protection, Inc.) for use in a tank mix with copper on field tomatoes, the long term effectiveness of this product under Ontario environmental conditions is not yet known.

A number of bacteria and fungi with a demonstrated ability to suppress the growth of plant pathogens have been screened for their ability to control field tomato diseases (Alabouvette et al., 2006; Pal and McSpadden Gardener, 2006). A number of pseudomonads including *Pseudomonas syringae* Cit7, *Pseudomonas fluorescens* A506 (BlightBan® A506, Nufarm Americas, Inc.), *Pseudomonas putida* B56 and *Pseudomonas gladioli* B25 have shown some promise for the management of bacterial spot (Byrne et al., 2005) and early blight (Jagadeesh and Jagadeesh, 2009), but, as with many biological control agents (BCAs), high levels of disease suppression under field conditions has not been consistent (Ojiambo and Scherm, 2006).

Streptomycetes are known for their production of antimicrobial metabolites, degradative enzymes, plant growth hormones and siderophores, as well as for their ability to suppress plant disease through the induction of local and systemic resistance (Schrey and Tarkka, 2008; Conn et al., 2008). Greenhouse studies have shown that *Streptomyces* spp. not only suppress fungal and bacterial diseases of tomato but also improve plant growth (El-Abyad et al., 1993; Sabaratnam and Traquair, 2002). Mycostop® (Verdera Oy, Kurjenkellontie, Finland) is a streptomycete-based (*Streptomyces griseoviridis* K61) biocontrol product registered for use in Canada and over 15 other countries against several root rot and wilt fungi, including *Pythium, Fusarium, Alternaria, Phomopsis, Phytophthora* and *Botrytis*, primarily on greenhouse crops. This bacterium has a number of different disease control mechanisms at its disposal; the one(s) it actually uses depends upon the target pathogen and environmental conditions (Lahdenpera, 2000). Another commercial disease control product with a streptomycete (*S. lydicus* WYEC108) as the active ingredient is Actinovate® SP (Natural Industries, Houston, TX); this product is registered in the US and Canada for foliar and soil-borne fungal diseases of greenhouse and field-grown crops. S. *lydicus* WYEC108 not only suppresses phytopathogenic fungi but also promotes plant growth and root nodule formation on peas (Tokala et al., 2002; Yuan and Crawford, 1995). There are no published reports on the effect of *S. griseoviridis* K61 or *S. lydicus* WYEC108 on early blight or anthracnose of field tomatoes.

The objective of the present study was to examine the possibility that *S. lydicus* WYEC108 and *S. griseoviridis* K61 would be of value in a biological control program focusing on bacterial spot, early blight and anthracnose in field tomatoes. A sustainable bio-based control strategy capable of simultaneously suppressing these three diseases would be appealing to both the organic and conventional tomato growers. Also included in this study was *P. fluorescens* A506, a strain registered in the US and Canada for suppression of fire blight of pome fruit and frost (USA only) on various field crops (including tomato). In an earlier study in which fifty biocontrol strains were rated for efficacy and consistency in reducing bacterial spot symptoms on greenhouse-grown tomato seedlings (Byrne et al., 2005), this strain scored as one of the top three. However, it provided limited protection against bacterial spot in field experiments (Byrne et al., 2005). It may have more value when combined with another BCA. Olivain et al. (2004) demonstrated that another *P. fluorescens*, strain C7, consistently improved the control of Fusarium wilt of flax when used in combination with the nonpathogenic *Fusarium oxysporum* strain Fo47. None of the published studies employing BCA combinations to control bacterial spot (Ji et al., 2006; Obradovic et al., 2005, 2009) have included the streptomycetes.

2. Materials and methods

2.1. Microorganisms, media and culture conditions

Alternaria solani JAT2265 was isolated from a naturally infected tomato fruit (cv. H9478) obtained from a field plot at the Southern Crop Protection and Food Research center in London, Ontario, Canada. It was identified based on the morphology of conidia obtained from pure cultures and inoculated fruit, in comparison with published descriptions (Domsch et al., 1980; Jones, 1991). Inoculum was prepared by incubating the freshly-isolated pathogen on V8 juice agar (Johnston and Booth, 1983) under fluorescent light (cool-white, approximately 600 lux) for 10–14 days at room temperature (22 ± 2 °C) and then gently scraping conidia from the plate surface and mixing them with sterile 0.1% (v/v) Tween 80 (10 ml/plate). The suspension was filtered through Miracloth and the conidial concentration was adjusted to log 5 conidia ml^{-1} using a hemocytometer. For long-term storage, the conidia were suspended in potato dextrose broth supplemented with 10% glycerol and placed in a −80 °C freezer.

Colletotrichum coccodes JAT2241 was obtained from the Greenhouse and Processing Crops Research center in Harrow, Ontario,