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Management of bacterial spot of tomato with phosphorous acid salts

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ABSTRACT

Phosphorous acid salts (PASs) were evaluated alone or in combination with other products for managing bacterial spot of tomato in greenhouse and field experiments in Florida during a 3-year period. Field treatments included a weekly schedule of PAS alone, PAS combined with standard copper-bactericide at full rate or half rate, PAS alternated with a standard copper-bactericide, and PAS every week plus biweekly applications of acibenzolar-*S*-methyl (ASM). Field data showed that disease control with PAS combined with standard copper-bactericide full rate, PAS alternated with a standard copper-bactericide, and PAS every week plus biweekly applications of ASM was similar to that obtained using the standard copper-bactericide program. In greenhouse experiments PAS alone had a similar level of disease compared to the standard program, whereas PAS combined with standard copper-bactericide full rate was significantly better than the standard program. Yields in the field experiments were not affected by any of these treatments. Phytotoxicity was observed when PAS was applied to the foliage of tomato seedlings under greenhouse conditions. These data suggest that PAS combined with standard copper-bactericide full rate and PAS plus ASM could be used for managing bacterial spot of tomato in the field in Florida. The mode of action of PAS is still unclear, as it only slightly affected multiplication of *Xanthomonas perforans in vitro*, and was also ineffective as a plant activator.

1. Introduction

Bacterial spot of tomato (*Solanum lycopersicum*) is one of the most devastating diseases on tomatoes in Florida (incited by two *Xanthomonas* species i.e., *Xanthomonas perforans* tomato races 3 and 4 and *Xanthomonas euvesicatoria* tomato race 1) (Jones et al., 2004) and other states in the Southeastern USA. Tomato is the number one vegetable crop in Florida, accounting for close to 65% of the fresh market production in the southern United States (Florida Tomato Committee, 2005). However, tomato yield and quality are threatened by bacterial spot, which continues to be economically important to the tomato industry in the southeastern United States (Pernezny et al., 1996; Bauske et al., 1998). Although promising management strategies have been developed recently, there are still continuing challenges and new opportunities to manage this disease in the tropical and subtropical environments that are favourable for the development of bacterial spot disease on tomatoes (Momol et al., 2002). In North Florida and South Georgia, traditional and current control strategies include weekly applications of copper products and copper plus mancozeb with biweekly acibenzolar-*S*-methyl (ASM; Actigard 50WG, Syngenta Crop Protection, Inc., Greensboro, NC) application, respectively. However, the control tactic is not effective when weather conditions are favourable for disease development, such as high temperature, high humidity and precipitation. Moreover, development of copper-resistant strains often occurs, which hampers the effectiveness of copper compounds (Marco and Stall, 1983; Ritchie and Dittapongpitch, 1991; Jones et al., 1991). Additionally, copper is hazardous to the environment, specifically copper toxicity to soil, water and human health (Flemming and Trevors, 1989; Santore et al., 2001). Increasing environmental concerns regarding copper application and its often ineffective disease control due to resistant bacterial strains, prompted a search for novel biorational approaches: either reducing the use of copper-bactericides or simply replacing them with low/reduced risk products for management of bacterial spot of tomato.

Phosphorous acid (H_3PO_3)-containing products, marketed commonly as fungicides, have been reported to be effective in control

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of diseases caused by oomycetes (Cohen and Coffey, 1986) and bacteria (Norman et al., 2006; Wen et al., 2007a). Studies on the control of diseases caused by oomycetes using phosphorous acid products have been well documented (Cohen and Coffey, 1986; Agostini et al., 2003; Johnson et al., 2004; Rebollar-Alviter et al., 2005). The mechanisms of phosphorous acid on diseases caused by oomycetes have been evidenced by both direct (inhibiting oxidative phosphorylation) and indirect action (stimulating the plant's natural defence response against pathogen attack) (Fenn and Coffey, 1985; Smillie et al., 1989; Biagro Western Sales, Inc., 2003; McGrath, 2004). However, reports on bacterial diseases are limited. The efficacy of phosphorous acid-containing products in control of bacterial spot has been evaluated in preliminary field experiments (Wen et al., 2007a), and K-PHITE (active ingredient: mono and dipotassium salts of phosphorous acid 53%, Plant Food Systems, Zellwood, FL) showed potential in the management of bacterial spot of tomato. Wen et al. (2007a) demonstrated that there was a direct effect of phosphorous acid salts (PASs) on the bacterial spot pathogen. However, it remained questionable whether or not it has an indirect effect.

Acibenzolar-*S*-methyl (ASM) has been reported to be effective in control of bacterial spot of tomato (Louws et al., 2001; Vavrina et al., 2004; Obradovic et al., 2004, 2005; Wen et al., 2007b) in greenhouse and field experiments. It is especially beneficial when integrated with copper-bactericide where copper-resistant populations predominate, a technique currently used by North Florida and South Georgia growers (Momol et al., 2002). Integration of ASM with resistant tomato genotypes has also been reported to be promising (Wen et al., 2007b). However, the efficacy of the combination of ASM with phosphorous acid products has never been examined.

The objectives of our research were to (1) test if PAS alone or in combination with the standard copper-bactericide was effective in controlling bacterial spot of tomato caused by *X. perforans* under greenhouse conditions; (2) investigate the antibacterial property of PAS on *X. perforans in vitro*; and (3) test if PAS alone or in combination with the standard copper-bactericide or ASM was effective in controlling bacterial spot of tomato caused by *X. perforans* under field conditions.

2. Materials and methods

2.1. Efficacy of PAS in control of bacterial spot disease on tomato under greenhouse conditions

2.1.1. Bacterial strains

A copper-sensitive race T3 strain of *X. perforans* (91-118) was used. The strain was stored in 30% glycerol at −80 °C. Before use, it was grown in nutrient agar plates at 28 °C for 24 h.

2.1.2. Plant material

In Quincy tomato cv. Solar Fire seeds were sown in flats (16 × 8 cells, cell size: 3.5 × 3.5 cm^2) containing growing medium (SunGro Metro-Mix 200 Series, Sun Gro Horticulture Canada Ltd), and watered daily in a greenhouse. Two-week-old seedlings were transferred to cells (SC10) of the Ray Leach Cone-tainer (Stuewe and Sons, Inc. Corvallis, OR) which were placed on trays RL98 (64 cm × 30 cm × 18 cm; 98 capacity; Stuewe & Sons, Inc. Corvallis, OR).

In Gainesville tomato cv. Bonny Best seeds were sown in 10-cm pots containing growing medium (Metro-Mix 300, Maryville, OK), watered daily and fertilized twice with a soluble 20–20–20 (N–P–K) fertilizer (0.4 g/pot; Peter's fertilizer Products, W.R. Grace & Co., Fogelsville, PA) in a greenhouse.

2.1.3. Bactericide treatments

In Quincy, treatments included (1) untreated control (UTC), (2) standard copper–mancozeb (1.80 g l^{-1} Kocide 3000 [DuPont, Wilmington, DE] equivalent to 1.68 kg l^{-1} label rate + 1.80 g l^{-1} of Manzate 75DF [DuPont, Wilmington, DE] equivalent to 1.68 kg ha^{-1} label rate) foliar applied 1-d prior to bacterial inoculation, (3) single foliar application of 0.75% K-PHITE applied 1-d prior to inoculation, and (4) two foliar applications of 0.75% K-PHITE 12- and 5-d prior to inoculation (using as a plant activator). Foliar spray was conducted using a handheld sprayer.

In Gainesville, treatments included UTC, standard control (half of the application concentration compared to Quincy but same schedule as in Quincy), 0.5% K-PHITE, and 0.5% K-PHITE plus a half rate copper–mancozeb foliar spray (1-d prior to inoculation).

2.1.4. Bacterial inoculation

In Quincy, four-leaf stage seedlings were inoculated with 10^8 cfu ml^{-1} suspension of *X. perforans* strain 91-118 in sterile distilled water by foliar spray with a handheld sprayer until runoff. Plants were then covered with plastic bags and placed in a growth chamber at 28 °C with a 12-h photoperiod for 48 h. Plastic bags were removed and the plants were transferred back to the greenhouse bench arranged in randomized complete block design.

In Gainesville, four-leaf stage seedlings were inoculated with a 10^7 cfu ml^{-1} suspension of *X. perforans* strain 91-118 in sterile tap water by foliar spray with a handheld sprayer until runoff. Plants were then covered with clear plastic bags and placed in a growth chamber at 28 °C with a 12-h photoperiod for 36–48 h. Plastic bags were removed and the plants were transferred back to greenhouse bench arranged in randomized complete block design.

2.1.5. Disease evaluation and data analysis

In Quincy, experiments were conducted twice. Five plants were used for each treatment. Two leaves per plant were assessed for bacterial spot disease severity 14-d after inoculation using Horsfall–Barratt scale (HB/leaf) as described by Horsfall and Barratt (1945). Each treatment had 10 replicates. Data were transformed to percent disease severity. Analysis of variance (ANOVA) and subsequent separation of sample means by the Waller–Duncan *K*-ratio *t* test (for balanced data) were carried out using SAS (version 9.1; SAS Institute, Inc. Cary, NC).

In Gainesville, experiments were conducted three times. Four plants were used per treatment. Two or three leaves per plant were assessed for bacterial spot disease severity 12-d after inoculation using HB scale, and then transformed to percent disease severity. Data analysis was subjected to ANOVA, and treatment means were compared using the Waller–Duncan *K*-ratio *t* test using SAS (version 9.1; SAS Institute, Inc. Cary, NC).

2.2. Antimicrobial activity of phosphorous compounds

X. perforans T3 strain 91-118 was prepared by transferring stock culture on nutrient agar plates, and incubating at 28 °C for 48 h. Bacterial inoculum was prepared by inoculating a loopful of cells from the active culture to 30 ml nutrient broth in a sterile 50-ml Falcon tube, and incubated at 28 °C overnight on an orbital shaker at 150 rpm. The overnight culture was centrifuged at 3000×*g* for 10 min, and the pellets were suspended in 20 ml of 0.01 M $MgSO_4$. Cell concentration was determined by measuring OD_{600}. PAS solutions (0.5% and 0.75% K-PHITE (V/V)) were prepared in nutrient broth (Difco Laboratories Ltd, Surrey, UK), and filter-sterilized by passing through 0.22 μm membranes. The pH values of solutions were measured. Fifty milliliters of 0.5% and 0.75% K-PHITE solutions and nutrient broth (as control) in a 125 ml flask were inoculated with 1×10^4 cfu ml^{-1} of the above prepared bacterial suspension, and incubated at 28 °C on an orbital shaker at 150 rpm. Bacterial populations were determined after 0, 3, 6, 24 and 48 h incubation by spreading appropriate dilutions prepared in 0.1% (W/V) peptone