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Evaluation of biocontrol agents and potassium silicate for the management of powdery mildew of zucchini

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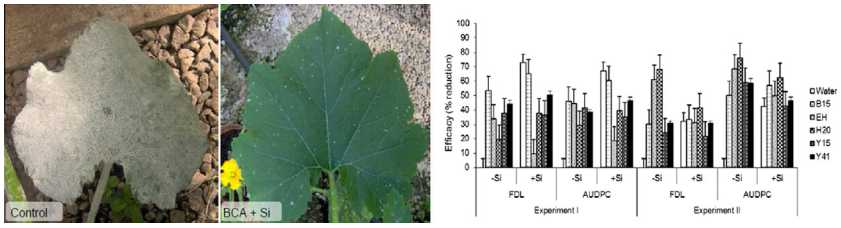
HIGHLIGHTS

- Biocontrol agents and silicon reduced the severity and AUDPC of powdery mildew.
- Temperature, RH and disease pressure affected efficacy of BCAs and Si.
- *Clonostachy rosea* and *Trichothecium roseum* performed well under extreme conditions.
- Application of K_2SiO_2 increased accumulation of Si in zucchini leaves.

GRAPHICAL ABSTRACT



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ABSTRACT

Investigations were conducted under greenhouse and field conditions to evaluate the effects of potential biocontrol agents (BCAs) and soluble silicon (Si) on powdery mildew of zucchini caused by *Podosphaera xanthii*. Five BCAs were applied as foliar sprays to zucchini leaves and Si was drenched weekly into the rhizosphere of these plants.

In the greenhouse, all BCAs provided significant control of powdery mildew with fungal isolates, reducing disease levels by up to 90%. Si alone reduced powdery mildew by as much as 35% and improved the efficacy of most of the biocontrol agents. Higher disease pressure reduced the efficacy of Si on powdery mildew but did not affect the performance of the BCAs. In the field, a disease reduction of 10–70% was achieved by BCAs and Si. Lower temperatures and high humidity ranges were suitable for optimal performances. The efficacy of the bacterial BCA, *Serratia marcescens* – B15 and silicon diminished at temperatures above 25 °C. The fungal BCAs (*Clonostachys rosea* – EH and *Trichothecium roseum* – H20) were better suited to higher temperatures (25–30 °C) and were tolerant of low RH values. Application of K_2SiO_2 to zucchini roots increased the level of Si in the leaves, which was responsible for suppression of the disease.

1. Introduction

Powdery mildew is one of the most widely distributed and destructive diseases of cucurbits worldwide (Pérez-García et al., 2009). Use of resistant cultivars and repeated application of fungicides have been the main practices used to control the disease. However, with resistant cultivars failing to meet commercially acceptable levels of protection, loss of fungicides due to the development of fungicide-resistant fungal mutants, and consumer rejection of pesticides, much recent research has focused on identifying environmentally-safe products that can replace or supplement conventional fungicides (McGrath and Shishkoff, 1999; Shishkoff and McGrath, 2002). The use of biocontrol agents (BCAs) for pow-

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dery mildew control has been considered among the safest options, providing some degree of success, especially under greenhouse conditions (Bélanger et al., 1995; Dik et al., 1998; Elad et al., 1996, 1998; Falk et al., 1995; Romero et al., 2007; Verhaar et al., 1996, 1999). Although such results have led to the development of commercial products (Elad, 2000; Elad et al., 1998; Ngugi et al., 2005), their efficacy is often restricted to specific environmental conditions which determine their establishment and biocontrol activities.

In our previous research, isolates of *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & Gams; *Serratia marcescens* (Bizio) and *Trichothecium roseum* were selected for their activity against *Podosphaera xanthii* in *in vivo* screening (Tesfagiorgis and Laing, 2010). Several strains of *S. marcescens* (Downing and Thomson, 2000; Roberts et al., 2007; Someya et al., 2000, 2003, 2005; Wu et al., 2012), *C. rosea* (Nobre et al., 2005; Sutton et al., 1997) and *T. roseum* (Huang and Kokko, 1993; Jayaprakashvel et al., 2010) have demonstrated their biocontrol activities against a wide range of pathogens. However, their performance under different environmental situations is unknown since most of these reports were based on investigations conducted under controlled conditions.

Silicon has been widely investigated for its ability to enhance growth and resistance of plants to biotic and abiotic stresses (Datnoff et al., 2007; Epstein, 2009; Ma and Takahashi, 2002). Adding Si into nutrient solutions has reduced powdery mildew of cucurbits grown hydroponically (Bélanger et al., 1995; Menzies et al., 1991a, 1991b; Samuels et al., 1991a; Schuerger and Hammer, 2003). Although the modes of action by which Si exerts its protective effects against diseases are complex (Epstein, 1999; Fauteux et al., 2005; Fawe et al., 1998; Ghanmi et al., 2004; van Bockhaven et al., 2013), there is a direct correlation between the ability of plants to absorb this element and the benefits they get from it (Dallagnol et al., 2009; Datnoff et al., 2007; Ma and Yamaji, 2006).

As a guideline, Bélanger et al. (1998) and Tesfagiorgis and Laing (2013) recommended that for optimal growth of the plant and fruit quality, Si should be applied at 100 mg L^{-1}. However, information on the level of Si accumulated by the plant when the element is supplied to the roots as a drench is not available. In addition, although potassium has an inhibitory effect on powdery mildew when applied as a foliar treatment in different forms (Reuveni et al., 1995, 1996), its role when supplied to plant roots in the form of potassium silicate is not known.

Objectives of this study were to evaluate the efficacy of these potential BCAs and Si against powdery mildew of zucchini caused by *P. xanthii* (Castagne) under greenhouse and field conditions, to determine the effects of prevailing temperature and relative humidity (RH) on powdery mildew severity and efficacies of BCAs and Si, to study the potential of using these two control options for an integrated disease management strategy, and to measure accumulations of Si and K in plant leaves in order to determine their role in powdery mildew control.

2. Materials and methods

2.1. Greenhouse experiments

2.1.1. Preparation of zucchini plants

Seedlings of zucchini squash (*Cucurbita pepo* L., F1-Hybrid Partenon) (Starke Ayres, South Africa), were raised in a greenhouse at a temperature of 26–28 °C and relative humidity (RH) of 75–85%. After producing two fully developed leaves, seedlings were transplanted into pots (180 mm in diameter) containing composted pine bark and transferred into another greenhouse (24–30 °C and 65–85% RH). The pots were kept at a distance of 50–60 cm within rows and 150–200 cm between rows and were irrigated with 0.5 g NPK (3:1:3) + 0.5 g $Ca(NO_3)_2$ (Ocean Agriculture (Pty) Ltd., Muldersdrift, South Africa) per liter by means of drip irrigation.

2.1.2. Preparation of P. xanthii and inoculation of zucchini plants

Inoculum of *P. xanthii* was obtained from naturally infected zucchini plants and maintained in a separate greenhouse (Tesfagiorgis and Laing, 2010). Conidia of *P. xanthii* were collected from the source plants following a previous technique (Askary et al., 1998; Dik et al., 1998). Within 2 h of collection, the conidial suspension was adjusted to 10^3 ml^{-1} using a hemocytometer and sprayed onto the leaves of zucchini seedlings with a hand sprayer (28/410 Trigger Sprayer, Blakelin Plastics, South Africa) at a rate of 5 ml per leaf.

2.1.3. Preparation of biocontrol agents

Three isolates of *S. marcescens* (Bizio) (i.e., Isolates B15, Y15 and Y41) and two fungi (*C. rosea* (Link) Schroers, Samuels, Seifert & Gams (syn. *Gliocladium roseum*) (Isolate EH) and *T. roseum* (Pers.) Link (syn. *Cephalothecium roseum*) (Isolate H20), previously isolated from powdery mildew of different plant species and selected for their activity against *P. xanthii* (Tesfagiorgis and Laing, 2010), were used in this study. All isolates of *S. marcescens* were inoculated into conical flasks (250 ml) containing 100 ml of nutrient broth (Merck Laboratory, South Africa), incubated in a shaker (GFL 1083, Labortechnik, Germany) at 28 °C and 150 rpm (rpm) for 72 h, and centrifuged at 9000 rpm for 15 min. The suspension was removed gently and the pellet was transferred into bottles containing sterile distilled water. Microbial suspensions of the two fungal BCAs were produced using cultures of these isolates on potato dextrose agar (PDA) (Merck Laboratory, South Africa), maintained at 25 °C. After 2 weeks of incubation, the mycelia and spores were harvested by pouring 5 ml of sterile distilled water onto the plates for 5 min and scraping the mycelial mat with a sterilized scalpel. The mycelial suspension was transferred into sterile bottles, shaken vigorously and spores were filtered through four layers of cheesecloth.

2.1.4. Application of biocontrol agents and silicon

The concentration of propagules per ml of each BCA was adjusted to 10^6 and 10^8 for the fungal and bacterial isolates, respectively. Break-Thru® (Universal Crop Protection, South Africa), a silicone wetting agent, was added to each microbial suspension at 0.25 ml L^{-1} and mixed thoroughly. All BCAs were sprayed onto the foliage of 2 weeks old zucchini seedlings 48 h prior to inoculation of the pathogen. The application of the BCAs was repeated after 4 days when the colonies of powdery mildew started to appear, and continued thereafter at 7 day interval for 5 weeks. Soluble silicon (250 ml at 100 mg L^{-1}), in the form of liquid K_2SiO_3 (K2550, PQ Silicas, South Africa), was drenched onto the roots of each plant 3 days before inoculation and continued weekly. Five applications were made at 7 day interval during the season. Where Si was not used as a treatment, the same volume of water without Si was drenched onto the rhizosphere.

2.2. Field experiment

The field experiments were conducted at Ukulinga Research Farm located at 29°40′ E and 30°24′ S, 715 m above sea level, in the Southern Tall Grassveld of South Africa (Morris, 2002) on heavy, deep soil of Bonheimer clays (Professor R. Melis[1], 2002, pers. comm.). The field was thoroughly plowed twice with a tractor and all the existing weeds were removed by hand. To moisten the soil and enhance survival of the seedlings, the field was overhead irrigated 1 day before transplantation and continued throughout the

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