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Efficacy of acibenzolar-S-methyl and two strobilurins, azoxystrobin and trifloxystrobin, for the control of corky root of tomato and verticillium wilt of eggplant

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Abstract

The efficacy of two fungicides, azoxystrobin and trifloxystrobin, and a plant defense activator, acibenzolar-S-methyl, for the control of tomato corky root and eggplant verticillium wilt, was evaluated under greenhouse and field conditions by means of dips, soil drenches or foliar sprays. The strobilurins provided excellent control of the two diseases under greenhouse conditions. Azoxystrobin was more effective than trifloxystrobin and reduced the severity of tomato corky root and eggplant verticillium wilt by 98% and 95%, respectively. Acibenzolar-S-methyl caused plant stunting and severe leaf chlorosis in the greenhouse experiments. In the field, azoxystrobin provided the best control. Tomato corky root severity was reduced by 83% and eggplant verticillium wilt severity by 29%. At the dosage used, acibenzolar-S-methyl was effective only in controlling tomato corky root. No phytotoxic symptoms were observed by any material in the field.

Keywords: Strobilurins; Azoxystrobin; Trifloxystrobin; Acibenzolar-S-methyl; ASM; Corky root; Verticillium wilt; *Pyrenochaeta lycopersici*; *Verticillium dahliae*; Chemical control; *Lycopersicon esculentum*; *Solanum melongena*

1. Introduction

Corky root of tomato (*Pyrenochaeta lycopersici* Schneider and Gerlach) and verticillium wilt of eggplant (*Verticillium dahliae* Kleb.) are severe diseases which can cause heavy yield losses (Polley, 1985; Ciccarese et al., 1994b).

Pyrenochaeta lycopersici attacks the parenchyma tissues of the root system, causing brown cortical lesions on main and secondary roots. The lesions become dark-brown, swollen and fissured. Severely affected plants show leaf yellowing, dwarfing and massive root failure while a number of adventitious roots are emitted from the crown (Ciccarese and Cirulli, 1983). The pathogen mainly survives in soil as microsclerotia formed within infected cortical tissues (White and Scott, 1973).

Verticillium dahliae penetrates plants through root tips or wounds on roots (Garber and Houston, 1966) and reaches the xylem. On eggplant, leaves show flaccidity, chlorosis and necrosis. Infected plants also show pronounced vascular discoloration, severe defoliation and stunting which leads to death of the plant. The pathogen survives in soil as highly persistent microsclerotia (Wilhelm, 1955).

An effective control measure against both pathogens is methyl bromide soil fumigation (Cirulli, 1968a; Jarvis, 1993), which is to be phased out by 2005. Although crop rotation slowly reduces inoculum density, it is not always profitable for intensive crops. Grafting plants on resistant rootstocks provides good disease control both of corky root (KNVF rootstocks) and verticillium wilt (*Ve*-tomato rootstocks), but high costs limit this control measure (Colella et al., 2001; Ioannou, 2001). Recently, soil solarization has provided promising results in controlling corky root and verticillium wilt (Colella, 1999; Ioannou, 2000; D'Amico, 2001; Ioannou, 2001).

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Partial control of these two diseases using biocontrol microrganisms has been reported in several studies (Ciccarese et al., 1994a; Colella et al., 2001; Narisawa et al., 2002), but commercial products are not yet available for growers.

Although resistant sources to *P. lycopersici* in tomato (Cirulli, 1968b, 1969; Hogenboom, 1970) and to *V. dahliae* in eggplant (Cirulli, 1968c; Nothmann and Ben-Yephet, 1979; Cirulli et al., 1990) have been found, no resistant cultivars are commonly available.

Chemicals other than fumigants have not been tested against corky root of tomato. The antifungal antibiotic aureofungin, and the fungicides benomyl, captan, carbendazim, thiram and others effectively reduced verticillium wilt of eggplant (Thorat et al., 1976). To date, strobilurins and acibenzolar-S-methyl (ASM) have not been tested in the tomato/*P. lycopersici* and eggplant/*V. dahliae* pathosystems.

Strobilurins are a relatively new class of fungicides originating from the natural compound strobilurin A, which is a secondary metabolite produced by the Basidiomycete *Strobilurus tenacellus* (Clough et al., 1995). They inhibit electron transport between cytochrome *b* and cytochrome c_1 in the mitochondrial respiratory chain, thereby disrupting the production of ATP. This new mode of action may prevent or delay the development of pathogen strains resistant to standard fungicides. Strobilurins have a broad spectrum of activity against fungal species belonging to Oomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (Anke, 1995).

ASM is perhaps the most potent synthetic systemic-acquired-resistance (SAR) activator discovered to date (Kessmann et al., 1994); it is a functional analogue of salicylic acid (SA) and elicits the same SAR pathway and accumulation of the same pathogenesis-related proteins (PRPs) as SA does (Friedrich et al., 1996). ASM elicits and/or potentiates plant defenses (Katz et al., 1998). Studies have shown that, under field conditions, ASM protects against a broad spectrum of pathogens in several crops (Görlach et al., 1996).

No fungicides or SAR activators are currently registered in Italy for the control of corky root of tomato and verticillium wilt of eggplant.

The present research was conducted in order to evaluate the effectiveness of two strobilurins, namely azoxystrobin and trifloxystrobin, and ASM for the control of corky root of tomato and verticillium wilt of eggplant.

2. Materials and methods

2.1. Greenhouse experiments

Experiments were conducted in a glasshouse maintained at 22–28 °C. The greenhouse tomato cv Camelia and eggplant cv Mission Bell seedlings, both highly susceptible to corky root and verticillium wilt, respectively, were grown in steam sterilised soil in polystyrol trays with 60 cells (45 mm diameter × 60 mm height). Tomato and eggplant seedlings 45 and 60 days old, respectively, were transplanted into 15 cm diameter clay pots containing steam sterilised soil artificially inoculated with *P. lycopersici* or *V. dahliae*.

Inoculum of *P. lycopersici* was prepared from 1 month-old colonies produced on potato-destrose-agar (PDA) at 19 °C. Colonies were fragmented in water using a Waring blendor for 2 min and filtered through a 38 μm sieve (ASTM series, Mesh/no. 400) in order to separate microsclerotia (in the sieves) from mycelium and to obtain a water suspension of microsclerotia. The number of microsclerotia per milliliter of suspension was evaluated by means of a haematocytometer.

Inoculum of *V. dahliae* was prepared on perlite. This was dispensed (800 ml/bottle) in glass bottles and autoclaved (121 °C, 1 atm) for 30 min twice at 24 h intervals. Before the second sterilisation, 250 ml bottle^{-1} potato-sucrose broth was added to each bottle and the screw cap was substituted with a cotton plug. The perlite was inoculated (50 ml bottle^{-1}) with a *V. dahliae* conidial suspension (1×10^6 conidia ml^{-1}) in water prepared by scraping the agar surface of 10 days-old colonies cultivated on PDA. Bottles were incubated at 24 °C for 30 days. The number of microsclerotia per gram of perlite was evaluated as follows: 1 g of colonized perlite was ground in a mortar with 10 ml of water and the suspension obtained was observed under microscope with a haematocytometer.

Five days before transplanting, the *P. lycopersici*, microsclerotia suspension or *V. dahliae* colonized perlite were thoroughly mixed with steam sterilised soil (1:1:1 sand:silt:clay) to give a concentration of 50 microsclerotia g^{-1} of soil.

The following chemicals were tested: azoxystrobin (Ortiva, 23.2% a.i., SC, Syngenta, Italy), trifloxystrobin (Flint, 50% a.i., WG, Bayer CropScience, Italy) and ASM (Bion 50 WG, 50% a.i., Syngenta, Italy).

Dosage of chemicals, application procedure and timing of treatments are reported in Table 1.

Plants were fertilized weekly with a 1‰ 20:20:20 (= N:P_2O_5:K_2O) commercial soluble fertilizer.

The experiments were established as randomized blocks with four replicates of 10 plants each.

Corky root severity was assessed on tomato 120 days after transplanting and expressed as percentage of diseased root surface. Eggplant verticillium wilt incidence was evaluated 80 days after transplanting and expressed as percentage of wilted leaves.

2.2. Field experiments

Field experiments were conducted in two localities, one near Valenzano (Bari) on field soil naturally infested with *P. lycopersici* (2 microsclerotia g^{-1}, estimated by means of the Shiskoff and Campbell, 1990 method), and the other near Policoro (Matera) on field soil naturally infested with *V. dahliae* (10 microsclerotia g^{-1}, estimated by means of an