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Reduction of Fusarium wilt in watermelon by *Pseudomonas chlororaphis* PCL1391 and *P. fluorescens* WCS365

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Summary. Fusarium wilt of watermelon (*Citrullus lanatus*) caused by *Fusarium oxysporum* f. sp. *niveum* is a devastating soil-borne disease that causes extensive losses throughout the world. Two known *Pseudomonas* biocontrol strains were used separately and in combination to assess their antagonistic effectiveness against *F. oxysporum* f. sp. *niveum* in pot experiments. *P. chlororaphis* PCL1391 significantly reduced disease severity. *P. fluorescens* WCS365 was less effective in disease suppression, while a combination of the two bacteria had intermediate effects. The biological control of Fusarium wilt with *P. chlororaphis* offers a potentially useful tool in an integrated pest management program to control Fusarium wilt of watermelon.

Abbreviations. *Fon*: *F. oxysporum* f. sp. *niveum*; PCL: *Pseudomonas chlororaphis* PCL1391; WCS: *Pseudomonas fluorescens* WCS365.

Key words: *Fusarium oxysporum* f. sp. *niveum*, integrated pest management

Introduction

Fusarium wilt is one of the most important diseases of watermelon worldwide. It is caused by *Fusarium oxysporum* Schlechtend: Fr. f. sp. *niveum* (E.F. Smith) W.C. Snyder & H.N. Hansen. Although the use of resistant rootstocks and cultivars provides some degree of protection from fusarium wilt, the development of new pathogenic races is a constant problem, and there are currently no commercially acceptable cultivars with adequate resistance to *F. oxysporum* f. sp. *niveum* (Ermstrom and Hopkins, 1981; Martyn, 1996). Fusarium wilt can be controlled by pre-planting soil disinfestation. However, due to the environmental and human health risks relating to excessive chemical control, it is necessary to find effective alternative means to control the fungus.

Biological control offers the potential for the effective and environmentally friendly control of Fusarium wilt. Non-pathogenic strains of *F. oxysporum* have been used to reduce Fusarium wilt in various crops (Alabouvette *et al*., 1993; 1998). In addition a number of *Pseudomonas* spp. strains have shown a varying capacity to control Fusarium wilt in various crop species (Weller, 1988; Alabouvette *et al*., 1998). There are also several reports indicating that disease control can be improved using combinations of biocontrol organisms (Pierson and Weller, 1994; Dekkers *et al*., 2000). However, there is no information about particular strains that provide control of Fusarium wilt of watermelon.

The bacterial strains *P. chlororaphis* PCL1391

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and *P. fluorescens* WCS365 sufficiently control tomato foot and root rot caused by the pathogenic fungus *F. oxysporum* f. sp. *radicis-lycopersici* (Chin-A-Woeng *et al*., 1998; Dekkers *et al*., 2000). *P. chlororaphis* PCL1391 produces the antifungal metabolite phenazine-1-carboxamide (PCN) which controls tomato foot and root rot (Chin-A-Woeng *et al*., 1998). The bacterial strain *P. fluorescens* WCS365 acts by inducing systemic resistance in the plant (Kamilova *et al*., 2005).

The aim of the present study was to assess the antagonistic features of *P. chlororaphis* PCL1391 and *P. fluorescens* WCS365 and their efficacy in controlling *F. oxysporum* f. sp. *niveum* in watermelon, in order to establish provisional guidelines for an effective biological control strategy in an integrated pest management system.

Materials and methods

Pseudomonas chlororaphis PCL1391 and *P. fluorescens WCS365* were kindly provided by Guido Bloemberg (Institute of Biology, Leiden University, The Netherlands). Bacteria were routinely grown in Petri dishes containing King's medium B (KB) with 1.8% agar. Inoculum was produced in KB liquid culture, at 26°C on a rotary shaker at 110 rpm overnight.

Fusarium oxysporum f. sp. *niveum* was isolated from infected watermelon plants, cv. Crimson Sweet, grown in an open field at the National Agricultural Research Foundation, Thessaloniki, Greece. This isolate was proved to be highly pathogenic to water melon after artificial re-inoculations. The race of the pathogen was not identified. Cultures were routinely grown on potato dextrose agar (PDA). Inoculum (conidia) was produced as previously described by Chin-A-Woeng *et al*. (1998) and was adjusted to 10^6 conidia ml^{-1}.

Watermelon seeds of the cultivar Crimson Sweet were surface-sterilized with 0.5% of sodium hypochloride and then coated with the biocontrol bacteria as previously described by Chin-A-Woeng *et al*. (1998). Watermelon seedlings were grown in beds and were regularly irrigated. Four groups of seeds were sown: 1, uncoated seeds (control); 2, seeds coated with *P. chlororaphis* PCL1391; 3, seeds coated with *P. fluorescens* WCS365; 4, seeds coated with both bacteria in equal volumes. Plants were grown in the nursery until they reached the second real leaf stage. Seedlings were transplanted to larger pots containing a mixture of sphagnum peat moss and perlite at a ratio of 10:1. During transplanting, a second application of the biocontrol bacteria was done by dipping the roots of the plants for 15 min in a suspension of 1×10^9 cfu ml^{-1}. Then the wilt fungus was applied by dipping the root system of the plants in the fungal spore suspension prepared as above. In this way the combined treatments (Table 1) were applied as follows: i) no fungus- or bacteria-inoculated plants (control), ii) plants inoculated only with the fungus (*Fon*), iii) plants inoculated with *P. chlororaphis* PCL1391 and then inoculated with *Fon* (*Fon*+PCL), iv) plants inoculated with *P. fluorescens* WCS365 and then inoculated with *Fon* (*Fon*+WCS), v) plants inoculated with *P. chlororaphis* PCL1391 and *P. fluorescens* WCS365 and then inoculated with *Fon* (*Fon*+PCL+WCS).

Table 1. Disease index of watermelon plants after inoculation (a.i.) with *Fusarium oxysporum* f. sp. *niveum* (*Fon*) alone and with *Pseudomonas chlororaphis* PCL1391 (*Fon*+PCL), *P. fluorescens* WCS365 (*Fon*+WCS), or both bacteria (*Fon*+PCL+WCS).

Treatments	Disease index		
	2 weeks a.i.	3 weeks a.i.	4 weeks a.i.
Control	1.0 a[a]	1.0 a	1.0 a
Fon	2.0 c	3.9 c	3.9 d
Fon+PCL	1.3 ab	2.3 b	2.7 b
Fon + WCS	1.4 b	2.8 b	3.8 d
Fon + PCL + WCS	1.3 ab	2.5 b	3.3 c

[a] Means within a column followed by the same letter are not significantly different at $P<0.05$ as determined with Duncan's multiple range test. Disease index scale: 1, apparently healthy plant; 2, slight chlorosis of lower leaves; slight wilt of plant; 3, necrosis, falling of lower leaves, yellow areas on the upper leaves; 4, dead plant.

Following treatment the plants were left to grow for 4 weeks at temperatures from 22 to 26°C and were regularly watered. The experiment was repeated three times and 20 plants per treatment were used.

Disease severity was estimated two, three and four weeks after transplanting, and was expressed according to a 4-point disease index scale based on progressive wilt symptoms. Analysis of variance was performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA) according to a completely randomized design. Significant differences were determined using Duncan's multiple range test at the $P<0.05$ level. The three replicates of the experiment yielded similar results. Values presented in the results are the mean value of these replicates.

Results and discussion

Disease severity was significantly but variously affected by the bacterial strains (Table 1). Two weeks after transplanting, the plants that had received only *Fon* had the highest disease index. The disease index was significantly lower when plants received *P. chlororaphis* PCL1391 as well as *Fon* (*Fon*+PCL). The disease index of plants receiving *Fon*+PCL, although slightly higher, did not differ significantly from the disease index of the non–treated control. The disease index of plants receiving the other two treatments (*Fon*+PCL+WCS) and *(Fon+WCS),* was equal or similar to that with *Fon*+PCL at this time. A significantly lower disease severity, as compared to treatment with *Fon* alone, was also observed three and four weeks after transplanting in plants treated with *P.* chlororaphis PCL1391 (*Fon*+PCL), and with the combination of *P. chlororaphis* PCL1391 and *P. fluorescens* WCS365 (*Fon*+PCL+WCS). With *Fon*+WCS disease severity was significantly lower only for the first three weeks after transplanting; after four weeks disease severity with antagonist strain was no different from that of plants treated with *Fon* without any antagonists.

The antagonistic bacteria reduced disease severity in watermelon especially at the early stages of plant and disease development. The progress of the disease was rapid after the second week of inoculation. After this time the bacteria did not inhibit watermelon wilt although they kept it at significantly lower levels.

With *P. chlororaphis* PCL1391 disease severity was reduced by 41% even though the disease pressure was very severe because of the large inoculum density of *Fon* applied to the watermelon seedlings. The level of control achieved was comparable to that with other rhizobacterial strains used to control Fusarium wilt in other crops (Liu *et al*., 1995; Raaijmakers *et al*., 1995; van Peer *et al*., 1995). In experiments using *P. chlororaphis* PCL1391 to control *F. oxysporum* f. sp. *radicis-lycopersici* on tomato, disease incidence was reduced by 45% two weeks after inoculation (Chin-A-Woeng *et al*., 1998). It must be emphasized that the *P. chlororaphis* PCL1391 used in the present study was isolated from tomato roots, for this reason a similar reduction in the disease of another host has to be considered important, and the potential of *P. chlororaphis* PCL1391 as a biocontrol agent in other host-pathogen systems should be investigated.

Disease severity with *P. chlororaphis* PCL1391 was lower than that with *P. fluorescens* WCS365 possibly because the biocontrol mechanisms were different; and a combination of the two biocontrol agents had an intermediate effect.

The use of *P. chlororaphis* PCL1391 to reduce the severity of Fusarium wilt in watermelon should be further investigated. The biological control of *Fon* using *P. chlororaphis* PCL1391 is a potential tool in an integrated pest management program to control fusarium wilt in watermelon. Despite high disease pressure this bacterium kept disease severity at low levels in young seedlings at early stages of the disease. Later on, protection by the bacterium diminished and although it still significantly lowered disease severity, extra control measures would be needed to protect watermelon from wilt for a longer period. A better use of the available control measures such as fungicides, cultural practices, fertilizers, biological agents, tolerant cultivars and rootstocks can improve wilt control and provide better protection of the environment and the public.

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