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Induced resistance in tomato plants against Fusarium wilt invoked by Fusarium oxysporum f.sp. dianthi

B.A.M. KROON, R.J. SCHEFFER[1] and D.M. ELGERSMA

Department of Molecular Cell Biology, Section of Plant Pathology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, the Netherlands

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Abstract

Tomato plants, susceptible to *Fusarium oxysporum* f. sp. *lycopersici*, were inoculated by immersing the roots in a conidial suspension of *F. oxysporum* f. sp. *lycopersici* race 1, *F. oxysporum* f. sp. *dianthi* race 2 or a mixture of both fungi. Plants inoculated with *F. oxysporum* f. sp. *lycopersici* showed disease symptoms after 2 weeks, whereas plants inoculated with *F. oxysporum* f. sp. *dianthi* or a mixture of both fungi remained symptomless for over 7 weeks, the duration of the experiment.

In another experiment root systems of plants were split and each half was separately inoculated. One half was firstly inoculated with *F. oxysporum* f. sp. *dianthi* or treated with water, followed after a week by a second inoculation of the other half with *F. oxysporum* f. sp. *lycopersici* or by a water treatment. The disease symptoms in the half firstly inoculated with *F. oxysporum* f. sp. *dianthi* were significantly delayed, compared to plants of which that half had been treated with water. Because *F. oxysporum* f. sp. *dianthi* reduced disease symptoms caused by *F. oxysporum* f. sp. *lycopersici* without any direct interaction with this pathogen, it is concluded that *F. oxysporum* f. sp. *dianthi* is able to induce resistance against *F. oxysporum* f. sp. *lycopersici* in tomato plants.

Additional keywords: *Fusarium oxysporum* f. sp. *lycopersici*.

Introduction

Pretreatment of plants with non-pathogenic microorganisms can reduce the severity of the disease caused by a Fusarium pathogen (Davis, 1968; Gessler and Kuć, 1982; Hillocks, 1986; Biles and Martyn, 1989). Treatment of plants with abiotic stimuli can also reduce disease symptoms (Anchisi et al., 1985; Bovio et al., 1987; Ferraris et al., 1987). This phenomenon is called induced resistance. Both induction and the protective effect are aspecific. The mechanism is indirect and mediated by the plant.

Protection against *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. in tomato plants has been obtained with non-pathogenic forms of *F. oxysporum*, but also with pathogens unrelated to *F. oxysporum* (Langton, 1969; Vanachter et al., 1978; Phillips et al., 1967; Verma and Allison, 1970; Alabouvette, 1990). Generally, protection was only partial and not permanent. Because a direct interaction between inducer and pathogen was possible in these experiments, fungal antagonism in the soil or on the root surface could not be excluded.

[1] Present address: Zaadunie B.V., Westeinde 62, Enkhuizen, the Netherlands

The present experiments describe the resistance against Fusarium wilt in tomato plants induced by *F. oxysporum* f. sp. *dianthi* (Prill. & Del.) Snyd. & Hans. In some experiments a direct interaction between the fungi was avoided by spatially separating both inoculants.

Materials and methods

Tomato plants and fungi. Tomato plants of the cultivars Moneymaker (C32) and Craigella (GCR26), both susceptible to *F. oxysporum* f. sp. *lycopersici*, were grown in a glasshouse at 22-24°C. Seeds of the tomato lines were obtained from the Glasshouse Crops Research Institute, Littlehampton (UK).

F. oxysporum f. sp. *lycopersici* race 1 (WCS 801) and *F. oxysporum* f. sp. *dianthi* race 2 (WCS 816) were maintained on potato dextrose agar (PDA). Conidial suspensions were obtained by culturing the fungus in Czapek Dox medium (Oxoid CM95) on a reciprocal shaker for 7 days at 25 °C. Mycelial fragments were removed by filtering through sterile glasswool. After washing in sterile water the conidial suspension was adjusted to the desired concentration.

Inoculations of plants. Four-week-old plants were uprooted and inoculated by immersing the roots for 10 minutes in a conidial suspension of *F. oxysporum* f. sp. *lycopersici* (10^7 conidia/ml or 5×10^6 conidia/ml), *F. oxysporum* f. sp. *dianthi* (10^7 conidia/ml), or a mixture of both fungi (10^7 conidia/ml). Control plants were treated with sterile water.

In the other experiments, plants were inoculated with *F. oxysporum* f. sp. *dianthi* (10^7 conidia/ml) or treated with water, followed after a week by a second inoculation with *F. oxysporum* f. sp. *lycopersici* (10^6 conidia/ml) or by a water treatment. This was performed in three different manners. (1) Roots of 4-week-old plants were inoculated. Before the second inoculation, the roots of inoculated and water treated plants were washed for 30 seconds in 20% H_2O_2, and then rinsed in water. (2) Stems of 6-week-old plants were inoculated 1 cm above the cotyledons by making a small incision with a razor blade through drops of 20 μl of a conidial suspension placed on the 3 main vascular bundles of the stem. The suspension was allowed to be sucked into the vessels. After a week the plants were inoculated by immersing the roots for 10 minutes in a conidial suspension. (3) Root systems and hypocotyledons of 3-week- old plants of the cultivar Moneymaker were split and each half was replanted in a separate pot. After 2 weeks one half (first half) of the root system was inoculated by immersing it in a conidial suspension of *F. oxysporum* f. sp. *dianthi* (10^7 conidia/ml) or in water. After another week the other half (second half) was inoculated with *F. oxysporum* f. sp. *lycopersici* (10^6 conidia/ml) or treated with water.

Disease index and vessel discolouring. Disease symptoms were assessed using a arbitrary disease index: 0: healthy, 1: incipient wilt, epinasty of some leaves, 2: wilting of some leaves, 3: yellowing and necrosis of leaves, wilting of all leaves, 4: yellowing and necrosis of most leaves, some leaves fallen, 5: plant dead.

Upon termination of the experiments the discolouring of the vessels was scored.

Isolation and characterization of fungi from plants. The fungi were isolated from

the inoculated plants by placing slices of the stem on PDA supplemented with streptomycin (0.02%) and penicillin (0.01%). The *Fusaria* growing out of the slices were characterized by their ability to form heterokaryons with *F. oxysporum* f. sp. *lycopersici* or *dianthi*, using nitrate non-utilizing (nit) mutants (Puhalla, 1985).

Statistical analysis. Disease indices were compared using the two-sample test of Wilcoxon (Snedecor and Cochran, 1980).

Results

'Craigella' plants root-inoculated with *F. oxysporum* f. sp. *lycopersici* showed disease symptoms 2 weeks after inoculation. Plants root-inoculated with *F. oxysporum* f. sp. *dianthi* or with a combination of both fungi remained symptomless for 7 weeks, the duration of the experiment (Figs 1 and 2a). The same results were obtained with 'Moneymaker' plants (Fig. 2b).

All plants showed vessel discoloration except for the plants inoculated with *F. oxysporum* f. sp. *dianthi* or with a combination of both fungi. No fungi could be isolated from stems of plants inoculated with *F. oxysporum* f. sp. *dianthi*. The fungi isolated from plants inoculated with both fungi turned out to be *F. oxysporum* f. sp. *lycopersici*, because nit mutants of these fungi were able to form heterokaryons only with nit mutants of *F. oxysporum* f. sp. *lycopersici*.

'Moneymaker' plants, root-inoculated with *F. oxysporum* f. sp. *lycopersici*, all showed disease symptoms irrespective of the first inoculation in the stem (data not shown).

Removing the inducing fungus *F. oxysporum* f. sp. *dianthi* by washing the roots in 20% H_2O_2 resulted in a slight phytotoxic effect. Other washing procedures (lower concentrations of H_2O_2 or NaOCl) which did not have a effect on the plants, were

Fig. 1. 'Craigella' 3 weeks after inoculation with *F. oxysporum* f. sp. *lycopersici* (left) or with a mixture of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *dianthi* (right).

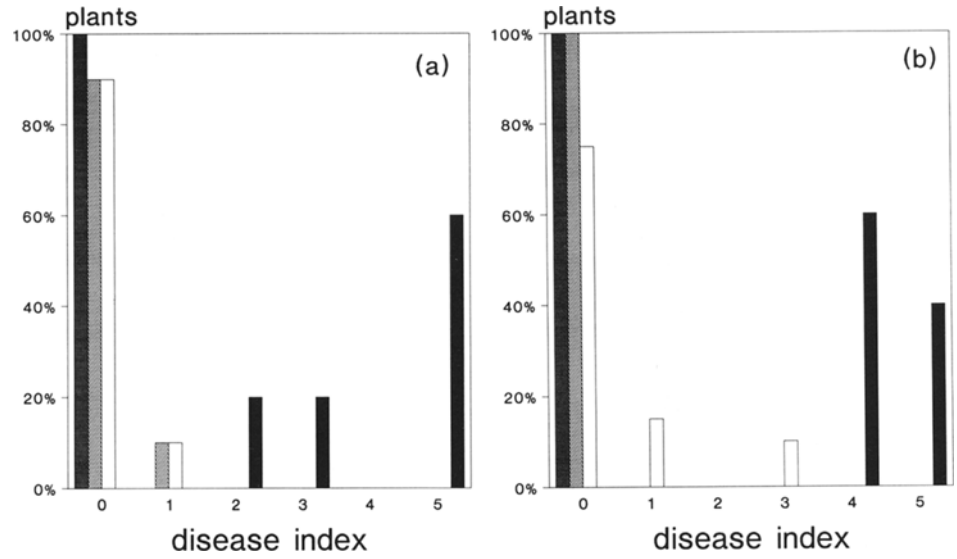

Fig. 2. Disease index of 'Craigella' (a) and 'Moneymaker' (b) inoculated with *F. oxysporum* f. sp. *lycopersici* (■), *F. oxysporum* f. sp. *dianthi* (▨) or both fungi (□), or treated with water (■). The differences in disease index between plants inoculated with *F. oxysporum* f. sp. *lycopersici* and plants inoculated with a mixture of both fungi were statistically significant (Wilcoxon, two sample test $\alpha < 0.01$).

not successful in killing the inducing fungus completely. However, the washing in 20% H_2O_2 also resulted in a statistically significant delay of symptom development in water treated plants after the second inoculation with *F. oxysporum* f. sp. *lycopersici* (Fig. 3).

Disease symptoms of plants with split root systems were scored separately for each half of the plant. Control plants (water treatments of both halves) and plants inoculated with *F. oxysporum* f. sp. *dianthi* followed by a water treatment, stayed healthy. Two weeks after the second inoculation the first disease symptoms became visible. The 'first half' of the plants showed less disease symptoms when this half was inoculated with *F. oxysporum* f. sp. *dianthi* than halves firstly treated with water (Figs 4 and 5). These differences in disease index were statistically significant from 19 days after the second inoculation until four weeks, the end of the experiment (Wilcoxon, two sample test, $\alpha = 0.05$). The 'second halves' of the plants, inoculated with *F. oxysporum* f. sp. *lycopersici* became more seriously diseased, but also a delay in symptom development was observed in the plants firstly inoculated with *F. oxysporum* f. sp. *dianthi* (Fig. 6). This difference was statistically significant from 19 days until 24 days after the second inoculation.

The same results were obtained when the second half was inoculated only 2 days after the first inoculation (data not shown). Only *F. oxysporum* f. sp. *lycopersici* could be isolated from stems of inoculated plants.

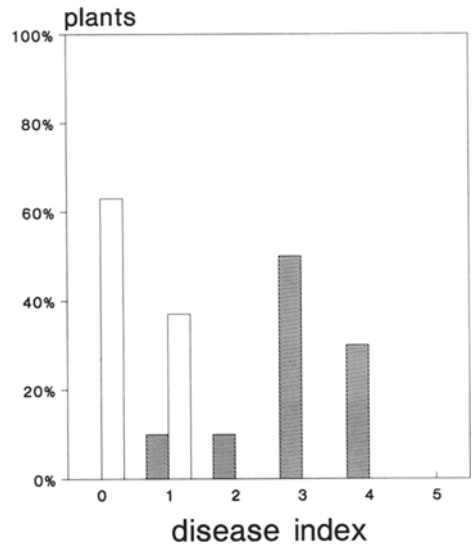

Fig. 3. Disease index of plants 3 weeks after inoculation with *F. oxysporum* f. sp. *lycopersici*. One week before inoculation the roots were treated with water. Before inoculation with the fungus the roots were washed for 30 sec with water (▒) or with 20% H_2O_2 (□). The differences in disease index were statistically significant (Wilcoxon, two sample test, $\alpha < 0.01$).

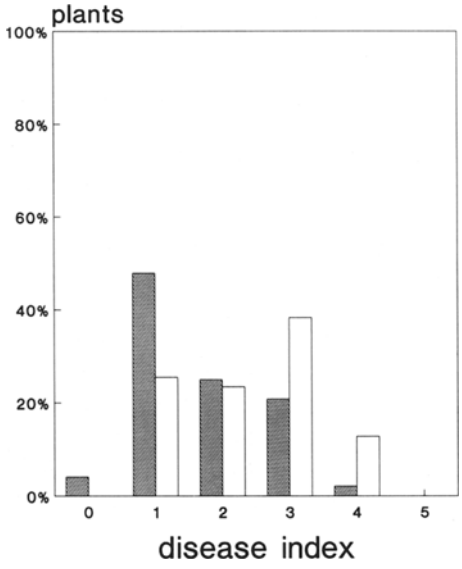

Fig. 4. Disease index of the first half of plants with split root systems, 3 weeks after the second inoculation. First inoculation: *F. oxysporum* f. sp. *dianthi* (▒) or water (□). Second inoculation: *F. oxysporum* f. sp. *lycopersici*. The difference in disease index was statistically significant (Wilcoxon, two sample test, $\alpha = 0.05$).

Fig. 5. Plants with split root systems 4 weeks after inoculation. First inoculation (pot 1): *F. oxysporum* f. sp. *dianthi* (left) or water (right). Second inoculation (pot 2): *F. oxysporum* f. sp. *lycopersici*.

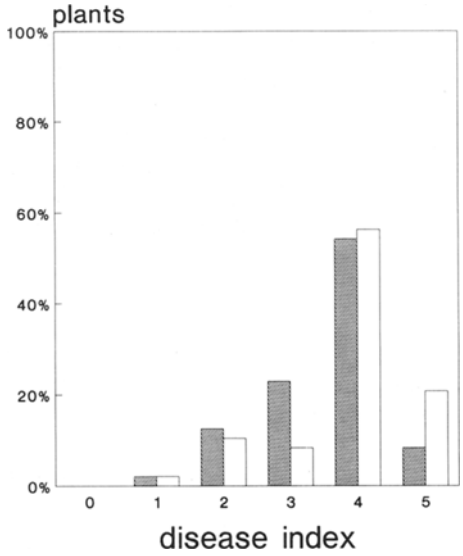

Fig. 6. Disease index of the second half of plants with split root systems, 3 weeks after the second inoculation. First inoculation: *F. oxysporum* f. sp. *dianthi* (▨) or water (□). Second inoculation: *F. oxysporum* f. sp. *lycopersici*. The difference in disease index was statistically significant (Wilcoxon, two sample test, $\alpha = 0.05$).

Discussion

In the experiments with mixed inoculations tomato plants were protected against Fusarium wilt by *F. oxysporum* f. sp. *dianthi*. In these experiments fungal antagonism could not be excluded. To study the role of induced resistance, competition between inducer and challenger, as observed by several authors (Alabouvette, 1990), must be avoided. Absence of fungal antagonism in vitro gives no evidence for the absence of a direct interaction in vivo (Matta, 1989). Maraite (1982) observed a delay of wilt symptoms of muskmelon, when two halves of root systems of seedlings were treated separately with inducer or challenger, but this delay was not important in the half treated with the challenger. In our experiments with tomato plants with split root systems, we also found a reduction of disease symptoms when the first half was inoculated with *F. oxysporum* f. sp. *dianthi*, but also in the half inoculated with the challenger, although the level of reduction of disease symptoms was not as high as in the experiments with mixed inoculations. Induced resistance is a plausible explanation for the observed suppression of symptom development, because antagonism was avoided by spatially separating inducer and challenger. Antagonism in the plant was improbable because only *F. oxysporum* f. sp. *lycopersici* could be isolated from the stem of the plant.

Inoculation of the stem with *F. oxysporum* f. sp. *dianthi* did not result in an induction of resistance. This observation and the fact that only *F. oxysporum* f. sp. *lycopersici* could be isolated from the stems of the plants which were inoculated with both fungi, indicates that induction of resistance is accomplished in the roots or on the root surface.

Anchisi et al. (1985) observed protection against Fusarium wilt in tomato plants after injuring the roots with hot water treatments. In our experiments treatment of roots with H_2O_2 also caused an induced resistance. Damage of the plants because of uprooting did not induce resistance, because water treated plants did not show resistance against Fusarium wilt.

The mechanism of induced resistance is unknown. Aspecific elicitors of *F. oxysporum* f. sp. *dianthi* or stress factors may induce defence reactions which are not suppressed by a blocker or suppressor produced by the pathogen. More knowledge about the kind of defence reactions in induced resistance and about the mechanism of induction is still to be gained.

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