Oxalic-Acid Elicited Resistance to Fusarium Wilt in *Lycopersicon esculentum* Mill.

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Abstract

Systemic induced resistance (SIR) in a plant enhances disease resistance to a broad spectrum of pathogens. Under climate chamber conditions, oxalic acid's ability to elicit SIR in tomato (*Lycopersicon esculentum* Mill.) against wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) was tested with a susceptible cultivar (Danish Export). Oxalic acid (OA) was sprayed onto the green part of the tomato plants, at concentrations 2.5, 5, 10, and 20 mM. Two days later, each plant was challenged with 10 ml of *Fol* suspension (10^6 conidia/ml) inoculated into the soil around the root system. After inoculation, disease incidence (DI) was quantified visually to assess SIR expression. OA-induced resistance (concentration-dependent) by otherwise susceptible tomato plants was obtained.

Keywords: oxalic acid; *Lycopersicon esculentum* Mill.; systemic induced resistance; *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*)

INTRODUCTION

Interest in the development of biological agents for use in control of plant pathogenic microorganisms has become worldwide. One fungal-plant system of wide interest in such development is the fungus *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) and the plant *Lycopersicon esculentum* Mill. (tomato). *Fol* causes tomato wilt, one of the most prevalent and destructive diseases of tomato, especially where it is grown intensively. *Fol* can cause great losses under suitable conditions, particularly in susceptible cultivars (AGRIOS 1997) – as a consequence of stunting and wilting, finally ending in death. In plants, systemic induced resistance (SIR) mechanisms against various pathogens by biotic and abiotic inducers has been reported for many plant-species (KUĆ 1995). Classical inducers include pathogens, chemicals, plant growth promotion rhizo-bacteria (PGPR), and plant products. The objective of this investigation was to determine whether oxalic acid (OA) could be used to induce resistance to *Fusarium* wilt in tomato plants, by spraying OA at different concentrations to the green parts.

MATERIALS AND METHODS

Plant material and growth

Tomato seedlings (21-day-old, 2–4 leaves) of cv. Danish Export (susceptible to race 2 of *Fol*) were grown in 10-cm diam. pots containing a non-sterile commercial peat mixture "Enhetsjord P" (GERHARDSON *et al*. 1985) mixed with sand (80:20). The potted plants were maintained in a greenhouse with a 12h photoperiod, day/night temperatures 26 ± 2°C/22°C, and RH 60–70%. Fifty ml of water-fertilizer solution (Osmocote Plus micro) was applied in the course of watering the tomato plants three times weekly.

Fungus

Strain 165.85 of *Fol* (race 2) was obtained from the Centraalbureau voor Schimmelcultures, Baarn, The

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Netherlands, and used in the experiments. *Fol* cultures were stored in potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI, USA) slants at 5°C and grown in darkness (20–25°C). Microconidial suspension was prepared according to ATTITALLA *et al.* (2001).

In vitro

Using the method of PADMODAYA & REDDY (1996), OA at concentrations 2.5 mM, 5 mM, 10 mM, and 20 mM was tested for ability to antagonize mycelial growth of *Fol* in cultures of five solid media: V8-juice agar (V8), potato dextrose agar (PDA), Malt agar (MA), Komada medium (KOMADA 1975), and cornmeal agar tetracycline (CMAT). Those same concentrations were used to test OA (same concentrations) was also tested in two liquid media, Czapek-Dox broth (Difco) and potato dextrose broth (Difco).

Assessment of SIR

The experimental trials were run using a randomized block design with 20 blocks. For induction of systemic resistance, each OA concentration was sprayed (using a hand sprayer) to a designated group of plants twice (to run-off) on the stem and leaves, with a 2- to 3-day interval between sprayings. Forty eight hours after applying OA to the green parts of plants, any SIR that may have occurred was challenged by soaking the root system of each plant with 10 ml of *Fol* suspension (soil treatment – 10^6 conidia/ml).

To assess SIR expression during a period of 120 days after challenge inoculation, disease incidence (DI) (i.e. percentage of diseased plants) was calculated by visual inspection. A DI value of 0 (i.e. 0%) indicated that no wilt disease was observed, and a DI value of 100 (i.e. 100%) indicated that all plants were diseased. Statistical analyses were conducted with Duncan's test ($P = 0.05$).

RESULTS AND DISCUSSION

None of the four concentrations of OA inhibit radial growth of *Fol* either in solid or liquid media used at *in vitro* experiments.

The effects of OA at different concentration levels on disease development caused by *Fol* (*in vivo*) are depicted in (Figures 1A–D). Results of this study confirm that resistance to tomato wilt caused by *Fol* on otherwise susceptible plants can be obtained by

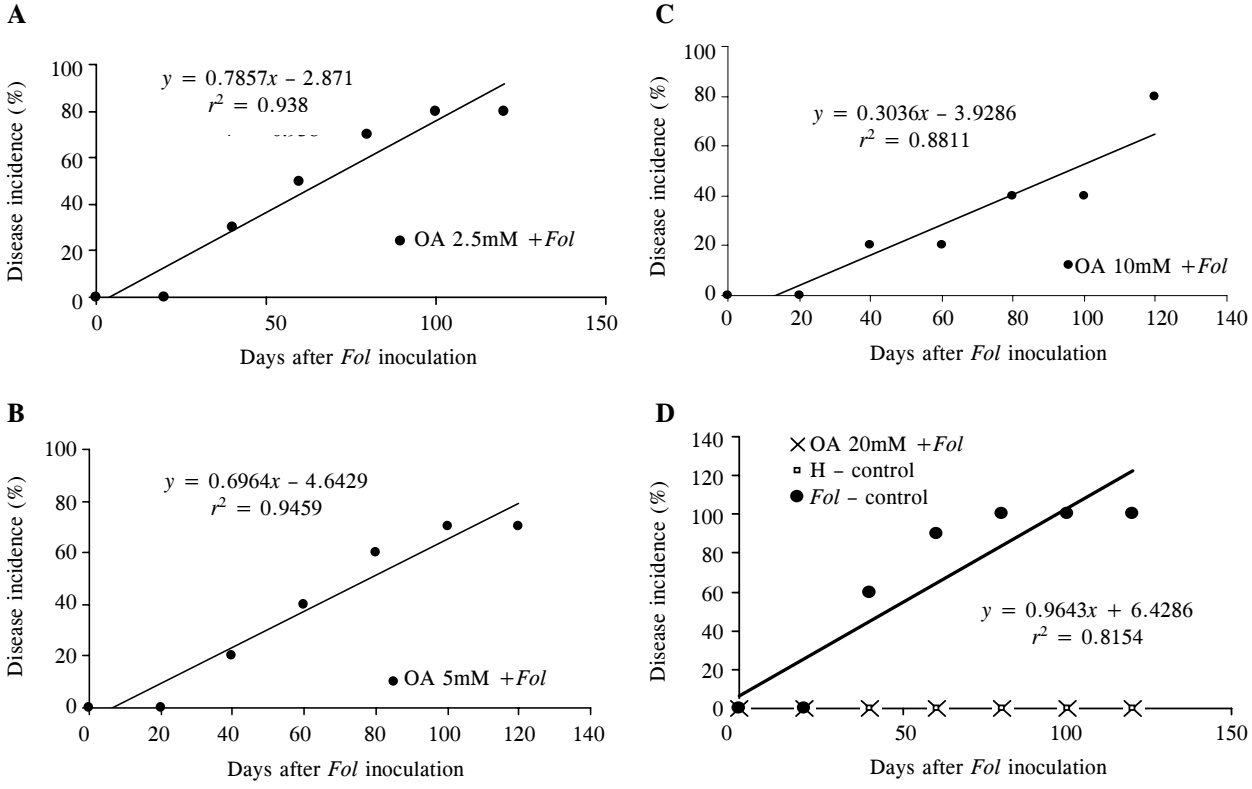

Figure 1. Effects of oxalic acid (OA) at different concentrations on incidence of wilt disease caused by *Fusarium oxysporum* f.sp. l*ycopersici* (*Fol*): (A): at 2.5mM; (B): at 5mM; (C): at 10mM; (D): at 20mM

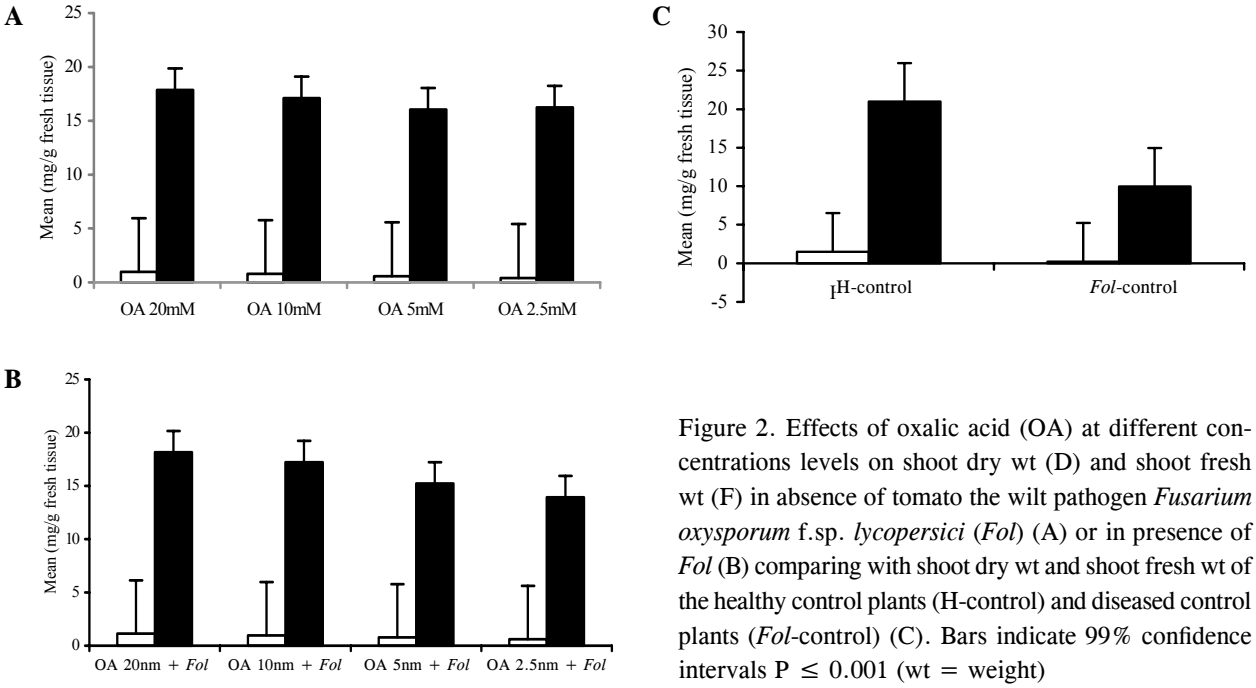

Figure 2. Effects of oxalic acid (OA) at different concentrations levels on shoot dry wt (D) and shoot fresh wt (F) in absence of tomato the wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) (A) or in presence of *Fol* (B) comparing with shoot dry wt and shoot fresh wt of the healthy control plants (H-control) and diseased control plants (*Fol*-control) (C). Bars indicate 99% confidence intervals $P \leq 0.001$ (wt = weight)

OA, and it is clearly OA-concentration-dependent. The resistance was evident as significant inhibition according to DI in the case of high dosage of OA 20mM. At lower concentrations 2.5 and 5mM there were no effects (but one cannot rule out SIR of some sort or at some level). On the other hand at 10mM OA, DI was reduced. *Fol* caused slight decrease on the fresh weight in all treated plants as well in the dry weight (Figures 2A–C).

The use of OA shows that a rather simple organic substance can be used for induction of resistance and that this compound does not need to be pathogen inhibiting itself.

We conclude that the induced resistance obtained is concentration-dependent and not pathogen-specific, since it could be possible to use OA as an inducer in other plant-pathogen systems (DESCAZO *et al*. 1990; MUCHARROMAH & KUĆ 1989, 1991; WEETE 1992; BASHIR *et al*. 1997; NABILA 1999; TOAL & JONES, 1999). This evidence supports the hypothesis that plants have the potential for resistance to many pathogens, and that defense mechanisms can be induced by stress as well as by infection. This conclusion is based mainly on the fact that a relatively high concentration of OA is required for an clear SIR expression.

References

AGRIOS G.N. (1997): Plant Pathology. 4th ed. Academic Press. NY.

ATTITALLA I.H., JOHANSSON P.M., BRISHAMMAR S., GERHARDSON B. (2001): *Pseudomonas* sp. strain MF30 suppresses Fusarium wilt of tomato *in vivo*. Phytopathol. Mediterr., **40**: 234–239.

BASHIR N., HASHMI M.I., JAMIL F.F. (1997): Induction of systemic acquired resistance by oxalic acid in Chickpea (*Cicer ariennum*) against *Ascochyta rabiei*. Pakistan J. Phytopathol., **9**: 18–20.

DESCAZO R.C., RAHE J.E., MAUZA B. (1990): Comparative efficacy of induced resistance for selected diseases of greenhouse cucumber. Canad. J. Plant Pathol., **12**: 16–24.

GERHARDSON B., ALSTRÖM S., RÄMERT B. (1985): Plant reactions to inoculation of roots with fungi and bacteria. J. Phytopathol., **114**: 108–117.

KOMADA H. (1975): Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protect. Res., **8**: 114–125.

KUĆ J. (1995): Induced systemic resistance – an overview. In: HAMMERSCHMIDT R., KUĆ J. (eds): Induced Systemic Resistance to Disease in Plants. Kluwer, Dordrecht: 169–175.

MUCHARROMAH E., KUĆ J. (1989): Oxalix acid, potassium biphosphate and potassium phosphate as inducers of systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Phytopathology, **79**: 1144.

MUCHARROMAH E., KUĆ J. (1991): Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber. Crop Prot., **10**: 265–270.

NABILA A.A. (1999): Effects of chemical and heat treatments of seeds on squash infection by cucumber mosaic virus (CMV). Assuit J. Agric. Sci., **30**: 193–206.

PADMODAYA B., REDDY H.R. (1996): Screening of *Trichoderma* spp. Against *Fusarium oxysporum* f.sp. *lycopersici* causing wilt in tomato. Indian J. Mycol. Plant Pathol., **26**: 266–270.

TOAL E.S., JONES P.W. (1999): Induction of systemic resistance to *Sclerotinia sclerotiorum* by oxalic acid in oilseed rape. Plant Pathol., **48**: 759–767.

WEETE J.D. (1992): Induced systemic resistance to *Alternaria cassiae* in sicklepod. Physiol. Mol. Plant Pathol., **40**: 437–445.