Factors Affecting Cross-Protection in Control of Fusarium Wilt of Tomato

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ABSTRACT

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Cross-protection by the introduction of *Fusarium oxysporum* f. sp. *dianthi* into host tissue of tomatoes (*Lycopersicon esculentum*) susceptible to *F. oxysporum* f. sp. *lycopersici* was successful; however, the biocontrol agent was only effective when it was applied a few days before a challenge inoculation with the pathogen. Protection (manifested as a reduction in symptom expression) was apparent after 24 days when *F. oxysporum* f. sp. *dianthi* was used as the biocontrol agent but not 34 days after the challenge inoculation. The inoculum density of the cross-protection agent required for biocontrol was approximately equal to or higher than that used in the challenge inoculation with the pathogen. The results suggest that the use of cross-protection for biocontrol of Fusarium wilt of tomato is not effective under greenhouse conditions.

Cross-protection is considered a means of biological control whereby a biocontrol agent induces resistance in a host rather than through the mechanism of direct antagonism to the pathogen (3). A host susceptible to a pathogen is treated with a microorganism (usually a nonpathogen) that induces resistance in that plant to the pathogen. Cross-protection phenomena have been reported in the biocontrol of many foliar, root and stem rot, and vascular wilt pathogens (4,5,11,16,21,22). Severity of Verticillium wilt is reduced by treating cotton (*Gossypium hirsutum* L.) plants with heat-killed conidia or mildly pathogenic strains of *Verticillium* spp. (4,19). Many fungi protect against Fusarium wilt pathogens, including *Fusarium solani* (Mart.) Appel & Wr. (14,17), *Acremonium* (= *Cephalosporium*) spp. (18), and formae speciales of *F. oxysporum* Schl. (6,7,13).

Because nonpathogenic *Fusarium* spp. are commonly found in plants (1) and these organisms are reported to be effective against pathogens in cross-protection (6,8,10,12), the Fusarium wilt diseases could provide a suitable system for the study of cross-protection as a potential control method. This study was attempted to determine the effectiveness of cross-protection for control of Fusarium wilt of tomato (*Lycopersicon esculentum* Mill.) and factors affecting this phenomenon.

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MATERIALS AND METHODS

A culture of the tomato wilt pathogen, *Fusarium oxysporum* Schl. f. sp. *lycopersici* race 1, was obtained from J. P. Jones (Agricultural Research and Education Center, University of Florida, Bradenton 33505). A culture of *F. oxysporum* Schl. f. sp. *dianthi* (pathogenic to carnation, *Dianthus caryophyllus* L.) was isolated from wilted carnation plants in a commercial greenhouse in Colorado. Stock cultures were maintained in soil and were used to obtain single-conidium isolates of the fungi prior to each experiment (20). Only single-conidium cultures between 6 and 14 days old were used in the experiments.

Seeds of tomato cv. Bonny Best susceptible to *F. oxysporum* f. sp. *lycopersici* were planted in plastic flats (52 × 32 × 6 cm deep) containing steamed potting soil (peat:perlite:soil, 1:1:3). The plants were grown in a greenhouse (30 C day, 24 C night), and seedlings were used 16–30 days after emergence when they had two to five fully expanded, true leaves.

Roots of the tomato seedlings were washed under running tap water, taking care to avoid extensively damaging the roots. Roots were suspended in bud-cell suspensions of *F. oxysporum* f. sp. *dianthi* for 5 min. The bud-cell suspensions were prepared by growing the fungus for 3 days in liquid culture (3.5 g of Difco Czapek-Dox broth, 0.1 g of yeast extract, and 100 ml of distilled water) in 250-ml Erlenmeyer flasks on a rotary shaker. The contents of the flasks were filtered through cheesecloth to remove mycelial fragments, and the resulting bud-cell suspensions were washed five times by centrifugation (5,000 rpm for 15 min) and resuspended in distilled water. The concentration of the washed bud-cell suspension (range of 10^5 to 10^7 cells per milliliter of water) was determined using a hemacytometer, and the desired concentration was achieved by diluting with distilled water. Roots of control seedlings were suspended in distilled water.

After treatment with the potential cross-protection agent or water (hereafter referred to as pretreatment), seedlings were dibbled into vermiculite contained in 25.4-cm-diameter plastic pots and grown in the greenhouse. After various times (usually 2 days), the seedlings were inoculated with *F. oxysporum* f. sp. *lycopersici* by removing them from the vermiculite and suspending the roots for 5 min in bud-cell suspensions prepared in the same manner as the nonpathogen (hereafter referred to as the challenge inoculation). Roots of control seedlings were suspended in distilled water for 5 min. In preliminary experiments, concentrations below 10^3 cells per milliliter never induced significantly more symptoms than uninoculated controls, and significant levels of disease severity were achieved with inoculum densities above 10^6 cells per milliliter.

This procedure resulted in four basic treatments used in each experiment. Two control treatments included plants that were pretreated with distilled water followed by treatment with distilled water at the time of the challenge inoculation (designated $H_2O \rightarrow H_2O$) and other plants pretreated with the cross-protection agent followed by treatment with distilled water (designated *dianthi* → H_2O). An inoculated control consisted of plants pretreated with distilled water followed by challenge inoculation with the pathogen (designated $H_2O \rightarrow$ *lycopersici*). The cross-protection test treatment consisted of plants pretreated with the nonpathogen followed by inoculation with the pathogen (designated *dianthi* → *lycopersici*).

Following the challenge inoculation, the plants were planted in potting soil in 15.2-cm-diameter plastic pots (one plant per pot). The pots were buried in gravel in a greenhouse bench heated with soil heating cables to maintain the soil temperature at 28–30 C.

Fusarium wilt symptoms began to appear 2–3 wk after the challenge inoculation. Disease severity was rated according to the leaf grade system (9) at 2- to 7-day intervals from the onset of

symptoms through 30–50 days following the challenge inoculation with *F. oxysporum* f. sp. *lycopersici*. Data, reported as mean disease ratings for each treatment, were analyzed with an analysis of variance. Duncan's multiple range test was used for separation of means.

At the conclusion of each experiment, plants were removed from the pots, and stem and root segments were surface-disinfested in 0.05% sodium hypochlorite for 3 min and plated on *Fusarium* selective medium (15). Presence and extent of vascular browning were recorded when the segments were cut. Three to 6 days after plating, presence of *F. oxysporum* f. sp. *dianthi* and/or *F. oxysporum* f. sp. *lycopersici* in each plant was determined by observation of the plates and transfer of the fungal colonies to potato-dextrose agar slants. Isolates were identified by comparison with original single-conidium cultures.

RESULTS AND DISCUSSION

Over a 2-yr period, numerous experiments were done to test various formae speciales of *F. oxysporum* for their ability to induce cross-protection in tomato and to determine factors that affect the cross-protection reaction. Success in obtaining biological control depended upon a number of factors, including the particular forma specialis of *F. oxysporum* used as the cross-protection agent and the rate at which it was applied. Also, biocontrol was not effective if the challenge inoculation with *F. oxysporum* f. sp. *lycopersici* occurred more than a few days after application of the inducing fungus.

These factors were incorporated into a typical experiment that utilized *F. oxysporum* f. sp. *dianthi* as the cross-protection agent (Fig. 1). Pretreatment of tomato seedlings with 5×10^6, 1×10^7, and 5×10^7 bud-cells of the nonpathogen per milliliter before inoculation with the pathogen at 1×10^7 bud-cells per milliliter resulted in cross-protection, ie, disease symptoms were significantly ($P = 0.05$) less severe than the inoculated control. Pretreatment of seedlings with 10^5 or 10^6 bud-cells per milliliter of *F. oxysporum* f. sp. *dianthi* did not result in protection. These effects were apparent 24 days after the challenge inoculation. Ten days later, however, there was no significant reduction of mean disease rating in any inoculated treatment. Symptom expression in seedlings pretreated with the inducing fungus followed by treatment with water was not different from seedlings dipped in water only. The presence of low levels of "disease" in uninoculated treatments was caused by natural senescence of the lower leaves.

In other experiments where tomato cuttings were pretreated with *F. oxysporum* f. sp. *dianthi* or another forma specialis of *F. oxysporum*, then rooted and inoculated, cross-protection did not occur. In fact, pretreated and inoculated plants sometimes had more severe symptoms than inoculated controls. This phenomenon was observed in experiments using tomato seedlings (Fig. 1). Plants pretreated with the nonpathogen at 10^5 bud-cells per milliliter and inoculated with the pathogen at 10^7 bud-cells per milliliter had significantly more disease than the inoculated controls when symptoms were assessed 24 days after the challenge inoculation (Fig. 1). In all experiments in which cross-protection was observed, there was a dosage-response effect related to the concentration of conidia of the inducing fungus with which the plants were pretreated. High concentrations of the inducing fungus approximately the same as or greater than that of the pathogen resulted in protection, whereas concentrations of the nonpathogen lower than that of the pathogen did not result in protection.

Also, when cross-protection occurred,

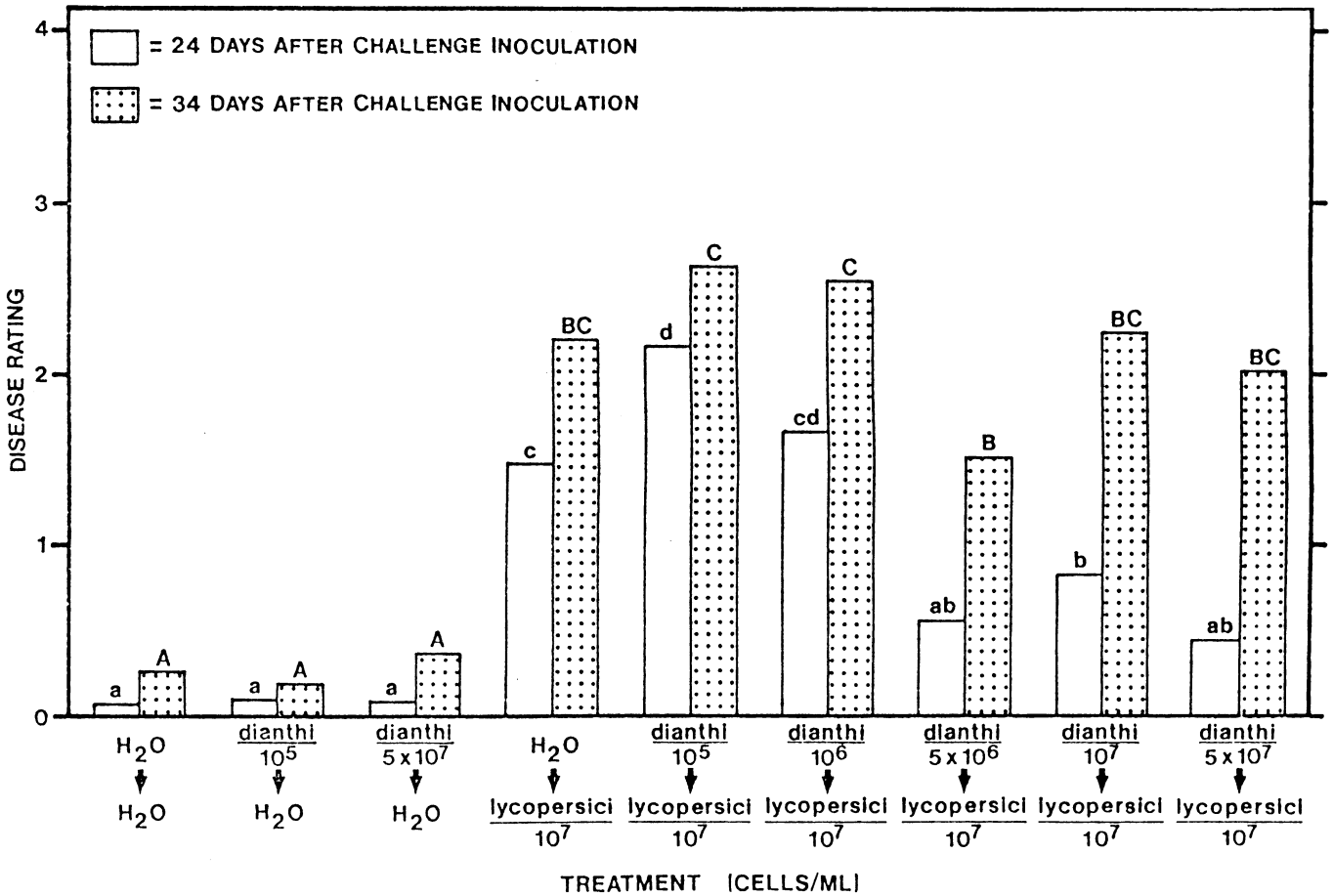

Fig. 1. Effect of pretreatment of tomato seedlings with water (H_2O) or with bud-cell suspensions of *Fusarium oxysporum* f. sp. *dianthi* on development of wilt induced by a subsequent challenge inoculation with water or with a bud-cell suspension of *F. oxysporum* f. sp. *lycopersici* race 1. Population density is given below each forma specialis. Challenge inoculation was 2 days after pretreatment. Bars represent mean disease rating (scale of 0–4) of 10 plants per treatment: 0 = no wilt symptoms, 4 = all leaves dead. Letters that are common over the same type of bar (solid or stippled) are not significantly different, $P = 0.05$.

it was expressed only as a delay in symptom expression. With sufficient time, wilt symptoms in protected plants were as severe as those in inoculated controls (Fig. 1).

Vascular browning was observed, and *F. oxysporum* f. sp. *lycopersici* was reisolated from all inoculated plants with symptoms; however, *F. oxysporum* f. sp. *dianthi* was not consistently reisolated from all plants previously treated with this fungus.

These results suggest that cross-protection in Fusarium wilt of tomato may be induced by the application of certain formae speciales of *F. oxysporum* that are not pathogenic to this host. The population density of the cross-protection agent, however, must be above a certain threshold level which, in these experiments, was approximately equal to or greater than that of the pathogen. Also, cross-protection occurred only when the challenge inoculation was attempted a few days after pretreatment with the inducing fungus but not later, confirming the work of Matta (13). Further, the cross-protection induced had only a temporary effect. For these reasons, the use of nonpathogenic formae speciales of *Fusarium* to induce a cross-protection response does not appear to be an effective or practical method for controlling Fusarium wilt of tomato under these conditions.

Why is the level of control with cross-protection so low and inconsistent for the vascular wilt pathogens in contrast with other plant disease systems? The mechanism of cross-protection in such systems is apparently related to the ability of the host to respond to a nonpathogenic entity by producing compounds inhibitory to the pathogen when it is subsequently introduced (2,4). Biocontrol agents, in those systems in which protection is substantial (3,5,11,14,22), are applied to living tissue; subsequently, the pathogens attempt to invade cells that are metabolically active and that have been induced to produce inhibitory compounds. In contrast, the Fusarium wilt pathogens largely penetrate through wounds or juvenile tissue (such as root tips) and invade nonliving vascular tissue. The only cells able to respond to a cross-protection agent are parenchyma that are intimately associated with the vascular elements, and massive amounts of the cross-protection agent may be required to induce inhibitory substances at these limited sites. This hypothesis predicts that cross-protection may only be efficient in plant disease systems where biocontrol agents and pathogens both interact in living tissue.

LITERATURE CITED

1. Armstrong, G. M., and Armstrong, J. K. 1948. Nonsusceptible hosts as carriers of wilt fusaria. Phytopathology 38:808-826.
2. Baker, R. 1968. Mechanisms of biological control of soil-borne pathogens. Annu. Rev. Phytopathol. 6:263-294.
3. Baker, R., Hanchey, P., and Dottarar, S. D. 1978. Protection of carnation against Fusarium stem rot by fungi. Phytopathology 68:1495-1501.
4. Bell, A. A., and Presley, J. T. 1969. Heat-inhibited or heat-killed conidia of *Verticillium albo-atrum* induce disease resistance and phytoalexin synthesis in cotton. Phytopathology 59:1147-1151.
5. Caruso, F. L., and Kuc, J. 1977. Protection of watermelon and muskmelon against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. Phytopathology 67:1285-1289.
6. Davis, D. 1966. Cross-infection in Fusarium wilt diseases. Phytopathology 56:825-828.
7. Davis, D. 1967. Cross-protection in Fusarium wilt diseases. Phytopathology 57:311-314.
8. Davis, D. 1968. Partial control of Fusarium wilt in tomato by formae of *Fusarium oxysporum*. Phytopathology 58:121-122.
9. Dimond, A. E., Davis, D. E., Chapman, R. A., and Stoddard, E. M. 1952. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomatoes. Conn. Agric. Exp. Stn., New Haven, Bull. 557. 82 pp.
10. Kaiser, S. A. K. M., and Gupta, P. K. S. 1977. Inhibition of wilt symptoms by *Fusarium oxysporum* f. sp. *udum* in pigeon pea (*Cajanus cajan*) induced by other formae speciales of *Fusarium oxysporum*. Phytopathol. Mediterr. 15:1-4.
11. Littlefield, L. J. 1969. Flax rust resistance induced by prior inoculation with an avirulent race of *Melampsora lini*. Phytopathology 59:1323-1328.
12. Matta, A. 1966. Effetto immunizzante di alcuni micromiceti verso le infezioni di *Fusarium oxysporum* f. *lycopersici* su Pomodoro. Ann. Fac. Sci. Agrar. Univ. Stud. Torino 3:85-98.
13. Matta, A. 1971. Microbial penetration and immunization of uncongenial host plants. Annu. Rev. Phytopathol. 9:387-410.
14. McClure, T. T. 1950. Fusarium foot rot of sweet-potato sprouts. Phytopathology 41:72-77.
15. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
16. Paxton, J. D., and Chamberlain, D. W. 1967. Acquired local resistance of soybean plants to *Phytophthora* spp. Phytopathology 57:352-353.
17. Perry, D. A. 1959. Studies on the mechanism underlying the reduction of pea wilt by *Fusarium solani* f. *pisi*. Trans. Br. Mycol. Soc. 42:388-396.
18. Phillips, D. V., Leben, C., and Allison, C. C. 1967. A mechanism for the reduction of Fusarium wilt by a *Cephalosporium* species. Phytopathology 57:916-919.
19. Schnathorst, W. C., and Mathre, D. E. 1966. Cross-protection in cotton with strains of *Verticillium albo-atrum*. Phytopathology 56:1204-1209.
20. Toussoun, T. A., and Nelson, P. E. 1976. *Fusarium*: A pictorial guide to the identification of *Fusarium* species according to the taxonomic system of Snyder and Hansen. Pennsylvania State University Press, University Park. 43 pp.
21. Weber, D. J., and Stahmann, M. A. 1966. Induced immunity to *Ceratocystis* infection in sweet potato root tissue. Phytopathology 56:1066-1070.
22. Yarwood, C. E. 1960. Localized acquired resistance to tobacco mosaic virus. Phytopathology 50:741-744.