Induction of Resistance to Fusarium Wilt in Cucumber by Root and Foliar Pathogens

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ABSTRACT

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Resistance to cucumber wilt (caused by *Fusarium oxysporum* f. sp. *cucumerinum*) was induced in cucumber plants growing on a mineral agar medium by inoculation of the medium with *F. oxysporum* formae speciales nonpathogenic on cucumber and by leaf infection with *Colletotrichum lagenarium* or tobacco necrosis virus (TNV). Resistance was not induced against the disease in plants growing in a synthetic soil mixture in a greenhouse by any of the fungi tested when challenge followed induction by 3 days or less. Resistance was induced by foliar infection with *C. lagenarium* or TNV, but not *F. oxysporum* f. sp. *melonis* when the interval was increased to 7 days.

Additional key words: Cucumus sativus.

Partial protection against Fusarium and Verticillium wilts induced by prior inoculation with pathogenic or nonpathogenic fungi or treatment with culture filtrates has been demonstrated in tomato and melon (5–8). The mycorrhizal fungus *Glomus mosseae* partially protected tobacco plants against disease caused by *Thielaviopsis basicola* (10) and tomato against Fusarium wilt (1,2). It also reduced infection by *Olipidium brassicae* in tobacco and lettuce, but only in roots infected by the symbiont. Mycorrhizal associations, however, enhanced disease caused by *Helminthosporium sativum* and *Erysiphe graminis* on barley, by *Colletotrichum lindemuthianum* and *Uromyces phaseoli* on French bean, by *Erysiphe cichoracearum* on cucumber, by *Botrytis cinerea* on lettuce, and by TMV on tobacco (11). Tjamos (12) reduced symptoms of Verticillium wilt in cucumber by treating the roots with culture filtrates of the fungus or by spraying the two basal leaves with a suspension of *V. albo-atrum* conidia.

In the present experiments, the induction of resistance against Fusarium wilt in cucumber by *Fusarium oxysporum* formae speciales nonpathogenic to cucumber, as well as by a virus and a fungal leaf pathogen, were investigated.

MATERIALS AND METHODS

Colletotrichum lagenarium Ell. & Halst. and tobacco necrosis virus (TNV) were maintained, and inoculum was prepared as described (3, and C. Gessler, *unpublished*). *Fusarium oxysporum* f. sp. *melonis* Snyder et Hansen, f. sp. *cucumerinum* Owen, f. sp. *lycopersici* Snyder et Hansen, and f. sp. *conglutinans* Snyder et Hansen were maintained on 2% malt agar. Microconidial suspensions or plugs from 7-day-old cultures were used as inoculum (C. Gessler, *unpublished*). Cucumber (*Cucumis sativus* L. 'Marketer') plants were grown at 24–27 C in 500-ml Erlenmeyer flasks on agar medium containing 0.005 M KNO_3, 0.0005 M $Ca(NO_3)_2$, 0.002 M $MgSO_4$, 0.001 M KH_2PO_4, 1 ml of iron tartrate solution (0.5%), and 1 ml of a trace element stock solution per liter. The trace element stock solution contained (mg/L): $Al_2(SO_4)_3 \cdot 18 H_2O$, 0.1; H_3BO_3, 0.5; $ZnSO_4 \cdot 7 H_2O$, 0.1; As_2O_3, 0.005; $BaCl_2 \cdot 2H_2O$, 0.03; $K_2Cr_2O_7$, 0.025; $Na_2MoO_4 \cdot 2H_2O$, 0.005; $MnSO_4 \cdot H_2O$, 0.35; and $CuSO_4 \cdot 5H_2O$, 0.05. Seeds were surface sterilized in 7% sodium hypochlorite for 10 min. Plants received 14 hr of light (10,600 lux [1,000 ft-candles] at leaf surface) per day from fluorescent tubes (Sylvania, cool-white, VHO, 215W) and supplementary incandescent bulbs. In the greenhouse experiments, plants were grown at 24–28 C in a synthetic soil mixture (Pro-Mix BX, Premier Peat Moss Corp., New York, NY 10036) as described earlier (4,9). The plants in flasks were inoculated with conidia of *C. lagenarium* by placing eight to ten 6.5-μl drops of a conidial suspension (10^6 conidia per milliliter) on the upper surface of each cotyledon. Plants were inoculated with TNV by placing ten 5-μl drops of inoculum from TNV-infected cucumbers on the cotyledons (3). The surfaces of cotyledons inoculated with TNV or treated with water were gently rubbed with a glass rod, which was flattened and roughened on the end, used to rub the cotyledons. In 3 days, 20 or more lesions were evident on the cotyledons infected with TNV. A plant treated with water (control) was included in each flask containing a plant inoculated with *C. lagenarium* or TNV. Inoculation with the *F. oxysporum* formae speciales in flasks was made by placing a 5-mm-diameter plug from malt agar cultures approximately 2 cm from the base of the seedlings. The second

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inoculation was made 5–10 days after the first.

To induce protection in greenhouse experiments, plants were inoculated on the first true leaf with thirty 5-μl drops of a conidial suspension of *C. lagenarium* (10^6 conidia per milliliter) (4) or inoculum from TNV-infected cucumber leaves (3). Inoculation with the Fusaria was made by pouring a microconidial suspension (50 ml, 5×10^6 or 10^7 conidia per milliliter) into the pots. Inoculation with *F. oxysporum* formae speciales, *C. lagenarium*, or TNV preceded inoculation with the pathogen by 3 or 7 days.

RESULTS

In three experiments with plants grown in flasks, all *F. oxysporum* formae speciales not pathogenic to cucumber completely protected cucumber against disease caused by subsequent challenge with *F. oxysporum* f. sp. *cucumerinum* (Table 1). Inoculation with *F. oxysporum* f. sp. *cucumerinum* killed all unprotected plants. Except for symptoms at the sites of induction on the cotyledons, plants inoculated with *C. lagenarium* appeared to be healthy in 75–100% of the cases, depending upon the experiment, and those inoculated with TNV all appeared to be healthy. The control in each flask was dead after 4–6 wk. In the same system, the protection against *C. lagenarium* by *C. lagenarium* was verified and found to be better than 90% based on lesion numbers. To the authors' knowledge this is the first report of resistance induced against a root pathogen by localized foliar infection with a fungus or a virus.

In one experiment, plants infected with *F. oxysporum* f. sp. *cucumerinum* were inoculated with *C. lagenarium*. The plants were not protected against *C. lagenarium* and had large foliar lesions 4–5 days after challenge even though some plants had started to wilt as a result of the infection with *F. oxysporum* f. sp. *cucumerinum*.

In other experiments, the medium in the flask was supplemented with 0.5% glucose or half the water in the medium was replaced with autoclaved or filter-sterilized 2% malt medium or autoclaved culture filtrate (2% malt medium) of *F. oxysporum* f. sp. *cucumerinum*. Protection was not induced by these additives, and protection was not induced by the nonpathogenic formae or *C. lagenarium* in the presence of the additives. The inducer and challenge fungi grew well on the medium containing the additives and covered its surface with white mycelial growth. In medium without an organic carbon source, the fungus covered the medium with only a thin net of growth.

Protection against Fusarium wilt was not induced by any of the fungi tested in plants grown in a synthetic soil mixture in a greenhouse when the time period between the inducing and challenge inoculation was 3 days (Table 2). *F. oxysporum* f. sp. *melonis* also did not induce resistance when the time interval was extended to 7 days. Resistance was induced in plants infected by *C. lagenarium* or TNV after a 7-day interval.

In another series of experiments, plants grown in the synthetic soil mixture treated with *Fusarium oxysporum* f. sp. *cucumerinum* or Fusaria nonpathogenic to cucumber were inoculated with *C. lagenarium*. The plants were not protected against *C. lagenarium* even when plants infected with *F. oxysporum* f. sp. *cucumerinum* were wilting 16 days after inoculation.

DISCUSSION

The results indicate that resistance induced in cucumber foliage against numerous foliar pathogens (3,4) is also effective in the roots against a root pathogen. As reported earlier for induced resistance in cucumber foliage (3,4), it appears that a time interval >3 days between induction and challenge is necessary for maximum protection. The inability to induce resistance in plants grown in the mineral medium supplemented with carbon sources suggests that the resistance can be overcome by high inoculum level or vigorous growth of the pathogen. In our work we evaluated the plants only after all diseased control plants had been dead for approximately 1 wk. Therefore, if a treatment reduced the rate of development of the disease but did not prevent its establishment, a protective effect would not be detected.

Experiments in flasks indicated that if only a part of the root system was in contact with *F. oxysporum* f. sp. *melonis*, the other part of the root system was completely protected when challenged with *F. oxysporum* f. sp. *cucumerinum*. This observation and the inability of *F. oxysporum* formae speciales to induce protection in the synthetic soil mixture argue against competition for infection sites between the pathogenic and nonpathogenic formae as a mechanism for resistance. Induction of resistance by the inoculation of foliage with *C. lagenarium* or TNV is obviously not

TABLE 1. Induction of resistance in cucumber plants growing on a mineral agar medium in Erlenmeyer flasks against *Fusarium oxysporum* f. sp. *cucumerinum*

Treatment		Dead plants (%) in experiment[b]		
First inoculation[a]	Second inoculation[a]	1	2	3
...	...	0	0	0
...	FOC	100	100	100
FOM	FOC	0	0	0
FOM	...	0	0	0
FOL	FOC	0	0	0
FOL	...	0	0	0
FOG	FOC	0	0	0
FOG	...	0	0	0
CL	FOC	0	25	12.5
TNV	FOC	0	0	...

[a] Inoculations were carried out by placing a plug in each flask with mycelium from *F. oxysporum* f. sp. *melonis* (FOM): *F. oxysporum* f. sp. *lycopersici* (FOL); *F. oxysporum* f. sp. *conglutinans* (FOG); *F. oxysporum* f. sp. *cucumerinum* (FOC) and by placing droplets of a conidial suspension of *C. lagenarium* (CL) or inoculum from TNV-infected plants on the upper surface of cotyledons. The surfaces of cotyledons inoculated with TNV were gently rubbed with a glass rod, which was flattened and roughened on the end, used to rub the cotyledons. Treatments with *C. lagenarium* and TNV included the controls in the same flask. Second inoculations were made 5–10 days after the first inoculation.

[b] Each number represents the mean of eight plants in the first and third experiment and six in the second except for experiments with *C. lagenarium* and TNV in which eight plants were used for each experiment.

TABLE 2. The effect of infection with *Colletotrichum lagenarium*, TNV, or *Fusarium oxysporum* f.sp. *melonis* on Fusarium wilt of cucumber plants grown in a synthetic soil mixture

Time between inoculation (days)	Treatment		Dead plants[c] (%)
	First inoculation[a]	Second inoculation[b]	
3			
	...	...	0
	...	FOC[b]	71
	CL	FOC	67
	TNV	FOC	63
	FOM	FOC	71
	FOL	FOC	63
	FOG	FOC	63
7			
	...	FOC	67
	FOM	FOC	58
	CL	FOC	13
	TNV	FOC	8

[a] The first true leaf was inoculated with either 30 5-μl drops of a conidial suspension of *C. lagenarium* (CL) or inoculum from TNV-infected (TNV) plants (3). Fifty milliliters of a microconidial suspension of the nonpathogenic *F. oxysporum* formae speciales (see Table 1) (10^7 conidia per milliliter) were poured into each pot. The first inoculation was made 3 or 7 days prior to the second inoculation.

[b] The second inoculation with *F. oxysporum* f.sp. *cucumerinum* (FOC) was made by pouring a microconidial suspension (5×10^6 microconidia per milliliter) into the soil.

[c] Data are the means from two experiments with 12 plants per treatment per experiment.

based on competition for infection sites. It is interesting that inoculating plants with the wilt pathogen, which does not elicit localized necrosis, prior to inoculation with *C. lagenarium* did not induce resistance against *C. lagenarium*.

The growth of cucumber plants in flasks on mineral agar provides a useful technique to study immunization under highly controlled conditions.

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