The Effect of Time of Exposure to Inoculum, Plant Age, Root Development, and Root Wounding on Fusarium Yellows of Celery

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ABSTRACT

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Fusarium yellows of celery was more severe as the time of exposure to inoculum of *Fusarium oxysporum* f. sp. *apii* in soil was increased from 4 to 14 days. Young, 2- and 4-wk-old, plants were more susceptible than were 6-and 8-wk-old plants. Root tips were important sites of infection, but wounded roots were not. Experiments in which celery roots were inoculated by the root dip method or the soil infestation method indicated that there was a finite number of susceptible infection sites (ie, apices of young roots) on celery roots at any one time, and that large numbers of infections were required for severe disease development. Subsequent disease severity was not as great after roots were dipped into inoculum as after roots grew through soil infested with the pathogen. This suggested that there was a limited number of infection sites during inoculation with the root dip method and that subsequently formed infection sites were not exposed to the inoculum.

The root dip method of inoculation of susceptible plants has been used successfully with most form species of *Fusarium oxysporum* when the inoculum consists of macroconidia (2,3,6,7,13,15,21). Inoculation of celery (*Apium graveolens* L. var. *dulce*) with *F. oxysporum* Schlect f. sp. *apii* (R. Nels and Sherb) Snyd. & Hans. by the root dip method was only partially successful; 25–50% of the inoculated plants escape infection and those that become infected frequently develop only mild symptoms (9). Dipping roots into inoculum in liquid agar (0.1%) and raising the inoculum concentration higher than 10^5 macroconidia and microconidia per milliliter did not increase infection or disease severity. The levels of infection and disease development are high only when celery plants are transplanted into soils artificially infested with *F. oxysporum* f. sp. *apii* (9).

This paper reports the relationship of plant age, root development, root wounding, and time of exposure to inoculum of *F. oxysporum* f. sp. *apii* to infection and disease severity of the fusarium yellows disease of celery.

MATERIALS AND METHODS

The susceptible commercial celery cultivar Tall Utah 52-70 R was used in this study. *F. oxysporum* f. sp. *apii* (FOA) isolate H171 was used for all of the experiments. Stock cultures of the fungus were maintained on autoclaved soil. The inoculum originated from these soil cultures and consisted of suspensions of macroconidia and microconidia scraped from the surface of potato-dextrose agar (PDA) plates after 1–2 wk of growth. Conidia were filtered through cheesecloth to remove mycelial fragments and the concentration was adjusted to 2×10^6 conidia per milliliter. Preliminary experiments indicated that washing conidia did not affect their infectivity or subsequent development. Therefore, unwashed conidia were used for subsequent experiments.

Inoculations were made by dipping roots of celery plants into conidial suspensions for 1–3 min and transplanting them into steamed UC soil mix, or by blending one PDA plate of FOA with 200 ml of sterile distilled water, mixed with 2 kg of steamed UC soil mix in a soil mixer, and the celery plants were inoculated by transplanting them into the infested soil. Celery plants grown in steamed UC soil mix, peat-sand (1:1 v/v), were carefully removed and transplanted to minimize wounding of the roots.

Disease ratings were recorded 30 or 60 days after inoculation and were based on the extent of vascular discoloration in the roots and crowns: 0 = no disease; 1 = vascular discoloration in one-half or less of the primary roots, and/or in several smaller roots, 2 = vascular discoloration in the primary root or other roots, extending up to the crown; 3 = vascular discoloration in up to 25% of the crown; 4 = vascular discoloration in 25–75% of the crown; and 5 = vascular discoloration in 76–100% of the crown. Dry weight of the aerial parts of infected and healthy celery plants was obtained in some experiments after the plant parts had been dried in an oven at 70 C for 3 days.

The effect of plant age on infection and disease development. Celery plants, 2-, 4-, 6-, and 8-wk-old, were transplanted into infested UC soil mix or naturally infested celery field soil for 4, 7, 14, 21, or 60 days. After each exposure period, the celery plants were removed from the infested soil, the roots were washed to remove adhering soil particles, and the plants were transplanted into steamed soil. Twenty plants of each age were tested at each exposure period, and all plants were harvested 60 days from the start of the experiments. Controls in noninfested soils were transplanted at the same time.

The relationship of wounding to disease development. Both the root dip inoculation technique (limited exposure to the inoculum) and the soil infestation technique (continual exposure to the inoculum) were utilized to determine the effect of wounding on disease development. Six-week-old celery plants with a minimal number of wounded roots at the time of inoculation were obtained by growing 4-wk-old plants for 2 wk in 100% modified No. 2 Hoagland's nutrient solution (10). Deionized water was added as needed and the fertilizer solution was changed every 2 days. In all, celery plants that had received 3 types of treatment were tested and inoculated by the root dip or the soil infestation technique: plants with minimally wounded roots grown in nutrient solution; plants with roots wounded naturally by uprooting, then grown in soil; and plants with severely wounded roots (75% of the roots removed) grown in either soil or nutrient solution. Twenty-five celery plants were inoculated per treatment and watered daily with dilute Hoagland's nutrient solution. Celery plants were harvested 30 and 60 days after inoculation by the soil infestation and root dip techniques, respectively. Control plants were treated the same, but were not inoculated.

The effect of time of exposure to inoculum on infection and

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TABLE 1. The effect of time of exposure to inoculum on disease development in Fusarium yellows of celery in artificially infested greenhouse soil and naturally infested field soil. The data are represented as the dry weight of inoculated plants as a percentage of the dry weight of uninoculated controls[a]

Inoculation period days	Plant age at inoculation (wk)							
	Greenhouse soil				Field soil			
	2	4	6	8	2	4	6	8
4	81.7	67.0	81.5	89.8	169.9	196.2	143.8	110.9
7	70.6	62.9	96.9	66.5	148.1	91.5	179.5	110.2
14	0.0	0.8	54.0	48.2	43.7	85.2	117.9	136.3
21	0.9	1.4	45.4	55.6	68.5	73.6	75.4	89.4
60	1.4	0.0	29.7	38.9	60.6	43.2	60.1	72.0
y-Intercept	55.2	47.6	80.8	73.01	129.1	135.4	152.0	121.0
Slope	−1.146	−0.998	−.953	−.653	−1.462	−1.765	−1.730	−.817
Significance[b]	.095	.006	.05	.054	.096	.007	.089	.328

[a] Inoculum consisted of PDA plates of *Fusarium oxysporum* f. sp. *apii*. Celery cultivar Tall Utah 52-70R was used in all experiments. Each number represents a total of four replications (five plants per replication) in two separate experiments.
[b] Significance level for H_0, $B = 0$.

TABLE 2. The effect of time of exposure to inoculum on disease ratings in different age celery plants infected with *Fusarium oxysporum* f. sp. *apii* in naturally infested field soil and artificially infested greenhouse soil[a]

Inoculation period (days)	Plant age at inoculation (wk)[b]							
	Greenhouse soil				Field soil			
	2	4	6	8	2	4	6	8
4	2.8	3.6	1.9	2.6	0.1	0.7	0.9	1.1
7	3.7	3.7	3.0	3.8	0.2	1.5	2.4	1.5
14	5.0	5.0	3.0	5.0	3.3	2.7	3.0	3.2
21	5.0	5.0	4.1	4.3	3.8	3.5	3.6	4.2
60	5.0	5.0	4.1	4.5	3.5	2.9	4.1	4.8

[a] Celery cultivar Tall Utah 52-70 R was used in all experiments. Each number represents a total of four replications (five plants per replication) in two separate experiments.
[b] Disease ratings scale: 0 = no disease, 5 = highest disease rating.

TABLE 3. The effect of different levels of root wounding and the method of inoculation on development of fusarium yellow of celery

Treatment[a]	Disease Rating[b]	Avg. dry weight (g/plant)	
		Inoculated	Control
Root dip inoculation			
unwounded	3.3 v	4.54 wx	5.01 wx
transplant wounds	4.0 v	4.27 xy	5.28 wx
pruned (i)	3.6 v	4.50 x	5.55 wx
pruned (ii)	3.7 v	5.06 wx	5.99 w
Soil infestation inoculation			
unwounded	4.6 v	2.12 z	4.43 x
transplant wounds	4.5 v	2.59 z	5.49 wx
pruned (i)	4.7 v	2.95 yz	5.37 wx
pruned (ii)	4.6 v	2.34 z	4.75 wx

[a] Six-wk-old cultivar Tall Utah 52-70 R plants were inoculated by the root dip or soil infestation methods of inoculation. Before inoculation with *Fusarium oxysporum* f. sp. *apii* one set of plants (i) was grown in steamed soil mix, the other set (ii) in nutrient solution. Roots were unwounded, wounded by normal transplanting, or severely wounded by removing ~ 75% of the roots. Dry weight and disease ratings of plants inoculated by soil infestation were recorded after 4 wk, and of those inoculated by the root dip method after 8 wk.
[b] Values in each column not followed by the same letters are significantly different ($P = 0.05$).

disease development. Six-week-old celery plants were transplanted into infested soil mix and exposed to the inoculum for 2, 4, 6, 8, 10, or 14 days. After each exposure period, the celery plants were removed from the infested soil, the roots were washed to remove adhering soil particles, and the plants were transplanted into steamed UC soil mix. Twenty plants were tested for each exposure time. Four weeks after the start of the experiment the plants were harvested and examined for signs of infection. Disease severity and dry weight were recorded and the number of discolored vascular bundles entering the crowns from infected roots was counted.

The relationship of new root formation to infection and disease development. Celery plants with different levels of new roots were obtained by transplanting 4-wk-old celery plants grown in soil into a nutrient solution or steamed soil as described previously. Twenty plants were inoculated by the root dip method 2, 4, 6, 8, 10, or 14 days after transplanting and were transplanted back into steamed UC soil mix. Disease ratings were recorded 60 days after the start of the experiment.

RESULTS

Plant age. In naturally and artificially infested soil, disease severity in 2-, 4-, 6-, and 8-wk-old plants increased as the time of exposure to the inoculum increased (Tables 1 and 2). The dry weight of infected plants was always less, as a percent of the controls, in artificially infested soil compared to naturally infested field soil. Celery plants in contact with the inoculum in infested soil for 14 days or longer generally had higher disease ratings and lower dry weights than did plants exposed to the inoculum for 4 or 7 days. This was especially true for 2- and 4-wk-old plants which were killed when grown in artificially infested soil. Similarly treated 6-and 8-wk-old plants were severely affected, but were not killed. In naturally infested soil, plants 2-, 4-, 6-, and 8-wk-old survived all exposure periods to the inoculum. Variations in dry weight of plants were much greater in plants grown in naturally infested soil than in artificially infested soil. The dry weight of some celery plants transplanted into naturally infested soil was greater than that of the controls.

The effect of root wounding. Disease severity (based on decreased dry weights) was greater in celery plants inoculated by the soil infestation method than in those inoculated by the root dip method (Table 3). This occurred even though plants inoculated by the root dip method were grown 4 wk longer. Compared to unwounded plants, wounding the roots prior to inoculation had no effect on the severity of disease development regardless of the degree of wounding or the method of inoculation. In all treatments, 100% of the celery plants were diseased.

The effect of length of exposure to the inoculum. As in the plant age experiment, the severity of fusarium yellows in 6-wk-old plants increased as the time of exposure to the inoculum increased (Table 4). The origin of discolored vascular bundles in the crowns of the 60 plants examined was from the vascular tissues of secondary roots attached to the crown. Colonization of the crown in regions other than through vascular tissues was not observed. As the length of exposure to the inoculum increased, the number of discolored vascular bundles entering the crowns through secondary roots increased.

The average number of discolored vascular bundles entering the crowns was 3.4, 5.8, 6.1, 10.3, 12.9, and 14.0 after 2, 4, 6, 8, 10, and 14 days of exposure, respectively (Table 4).

The effect of new root formation on infection and disease development. Vascular discoloration was less extensive (although

TABLE 4. The effect of time of exposure of celery cultivar Tall Utah 52-70R plants to inoculum of *Fusarium oxysporum* f. sp. *apii* on disease ratings, dry weight and the number of discolored vascular bundles entering the crowns[a]

Inoculation period (days)	Disease rating[b]	Dry weight[c] (%)	Discolored vascular bundles at crowns (no.)
2	1.6	106.3	3.4
4	2.9	86.0	5.8
6	2.9	79.6	6.1
8	3.8	85.5	10.3
10	4.3	71.7	12.9
14	4.4	63.8	14.0
y-Intercept	1.645	104.6	1.738
Slope	.220	−3.071	.954
Significance[d]	<.0001	.032	<.0001

[a] Each number is the average of two experiments, two replications per experiment (five plants per replication).
[b] Disease ratings scale: 0 = no disease, 5 = highest disease rating.
[c] Dry weight percentage is determined from the equation ([dry weight of infected plants]÷[dry weight of control plants]) × 100.
[d] Significance level for H_0, B = 0.

not significantly so), for celery plants grown in Hoagland's solution than for those grown in steamed UC soil mix (Table 5). Vascular discoloration remained low in plants grown in soil or solution culture for 2, 4, 6, or 8 days before root dip inoculations with FOA. The extent of vascular discoloration increased significantly (P = 0.05) in plants inoculated after 10 days of growth in soil and 14 days in solution culture. Increased disease severity was correlated with the initiation of new roots which emerged from the hypocotyl region after 6–10 days of growth in soil or solution culture. Also, the percentage of infected celery plants increased after 10 days of growth in soil or solution culture (Table 5).

DISCUSSION

We concluded that severity of fusarium yellows of celery increased as the time of exposure to inoculum increased because multiple infections were required for severe disease development. Since the maximum number of infections of discolored vascular bundles occurred at the time new roots were formed on transplanted plants (8–10 days) this suggests that infection occurs primarily through the apices of younger roots. Also, since wounding did not increase disease severity it can be concluded that wounded roots were seldom sites of infection. These two findings help to explain why disease severity was mild following root dip inoculations (ie, few unwounded apices of young roots were available as infection sites because most roots were wounded during transplanting). In addition, the inoculum in the root dip technique was present only on the inoculated roots; whereas, in the soil infestation technique the inoculum was present throughout the soil mass where it could come into contact with the apices of newly formed, elongating roots. Roots developed faster and more abundantly on celery plants grown in soil, which may explain why disease development was greater on these plants. These results agree with those of other research workers who report that root tips of hosts of other pathogenic species of *Fusarium* stimulated germination of conidia (3–5, 8, 14, 17) and were sites of infection (1,3,11,12,18–20).

The results of these experiments suggested that the pathogenic capability of *F. oxysporum* f. sp. *apii* was low, or that Tall Utah 52-70 R possessed resistance. However, of approximately 150 lines of celery exposed to the same *Fusarium* isolate in the greenhouse, Tall Utah 52-70 R ranks consistently among the most susceptible (16).

TABLE 5. The effects on infection and disease severity of growing celery plants in solution culture or soil prior to inoculation by the root dip technique with *Fusarium oxysporum* f. sp. *apii*.

Preinoculation period (days)[a]	Disease ratings		Infected plants (%)	
	Soil	Soln. Cult.	Soil	Soln. Cult.
0	2.4 [b]w		75 [b]xy	
2	2.0 w	1.4 w	70 y	50 z
4	2.2 w	1.3 w	80 x	60 yz
6	1.8 w	0.7 w	55 z	35 v
8	1.9 w	1.9 w	75 xy	70 y
10	4.0 x	2.7 w	100 w	90 wx
14	4.3 x	3.9 x	100 w	100 w

[a] Inoculum consisted of suspensions of macroconidia and microconidia (2×10^6/ml). Four-week-old celery plants of cultivar Tall Utah 52-70 R transplanted into steamed soil mix or Hoagland's nutrient solution.
[b] Values in each column not followed by the same letter are significantly different (P = 0.05). Disease rating scale: 0 = no disease, 5 = highest disease rating.

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