Interaction of *Erwinia chrysanthemi* and *Fusarium solani* on Sweetpotato

V. DUARTE, Former Graduate Student, and C. A. CLARK, Professor, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge 70803-1720

ABSTRACT

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Stem cuttings of sweetpotato cultivars Jewel and Beauregard were inoculated with *Erwinia chrysanthemi* and/or *Fusarium solani* and then transplanted in the greenhouse or the field. In the greenhouse tests, stem rot lesions in plants coinoculated with both pathogens were longer than those in plants inoculated with either pathogen separately. In the field tests, however, stem rot incidence was low, and no interaction effect was observed with either cultivar in 1989 or 1990. Neither pathogen, alone or in combination, had a consistent effect on yield. When the pathogens were applied to wounded storage roots, lesions were not significantly larger in coinoculated roots than in roots inoculated with *E. chrysanthemi* alone.

Erwinia chrysanthemi Burkholder, McFadden, and Dimock causes bacterial stem and root rot on sweetpotato (*Ipomoea batatas* (L.) Lam.) (3,7,12), and *Fusarium solani* (Mart.) Sacc. causes Fusarium root and stem canker (2,3, 9,11). Both diseases have two phases: a stem rot, which develops in the field, and a root rot, which affects storage roots in the field or in storage (3). Inoculations of sweetpotato vine cuttings with *E. chrysanthemi* or *F. solani* in separate experiments have failed to induce the severity of stem rot sometimes observed in the field (4,8). Because both pathogens affect the same plant parts and are transmitted through vegetative propagation of the crop, it is highly likely that they often occur together in plants. Sweetpotato vines and storage roots with symptoms of both diseases at the same time have been observed on occasion (C. A. Clark, *unpublished*). Many accounts in the literature suggest that *Fusarium* spp. increase the severity of blackleg or soft rot caused by *Erwinia* spp. on potato (*Solanum tuberosum* L.) (1,6,10,13–15).

Sweetpotato cultivars vary in susceptibility to both *F. solani* and *E. chrysanthemi* (4,5). The most widely grown cultivars in the United States are Beauregard and Jewel. Storage roots of Beauregard are considered susceptible to *E. chrysanthemi* and resistant to *F. solani,* while vines are resistant to both pathogens (4,5). Storage roots of Jewel are moderately resistant to both pathogens, while vines are resistant to *E. chrysanthemi* and have a low level of resistance to *F. solani* (4,5). These assessments, however, are based on inoculations with each pathogen separately, and the effect of coinfection with both pathogens is not known. This study was conducted to determine whether *E. chrysanthemi* and *F. solani* interact on the predominant sweetpotato cultivars.

MATERIALS AND METHODS

Inoculum. Strain Ech-2 of *E. chrysanthemi* and isolate M-10 of *F. solani* were used throughout this study. Both were isolated from sweetpotato and obtained from J. W. Moyer (Department of Plant Pathology, North Carolina State University). Both isolates were selected for use in earlier studies (4,5,8,9,11) because they were the most virulent isolates available. They were maintained on silica gel crystals at −20 C and were revived when needed. Before each experiment, pathogenicity was reconfirmed by inoculation of slices from storage roots.

Nutrient broth cultures (24 hr old, 32 C) of *E. chrysanthemi* were diluted in sterile distilled water (SDW) to an optical density of 0.15 at 620 nm (about 10^8 cfu/ml). Five-day-old potato-dextrose agar cultures of *F. solani* were washed with SDW and filtered through four layers of cheesecloth to obtain suspensions containing both macroconidia and microconidia. The concentration of conidia was determined with a hemacytometer and adjusted to 10^6 per milliliter.

Stem inoculations. Terminal vine cuttings (20–30 cm long) of Jewel (greenhouse tests 1 and 2) and Beauregard (test 2 only) sweetpotato were cut from plants in the field. Expanded leaves and petioles were removed, and the cut bases of the vines were dipped immediately into inoculum of *E. chrysanthemi,* inoculum of *F. solani,* inocula of both pathogens at the same concentrations as for single inoculations, or SDW. The cuttings were planted five per pot in 15-cm-diam clay pots filled with a 1:1 (v/v) mix of autoclaved river silt and sand. Five replicate pots were arranged in the greenhouse in a completely randomized design.

These experiments were conducted without supplemental lighting during June–August. Daily high temperatures in the greenhouse were 29–38 C, and low temperatures were 21–27 C. Pots were watered weekly with a fertilizer solution containing (per liter) about 0.37 g each of NO_3, P_2O_5, and K_2O. Six weeks after inoculation, roots were washed free of soil, stems were cut longitudinally, and the length of stem rot lesions was measured. Plants were then dried for 48 hr at 60 C and weighed.

For the field tests, stem cuttings of Jewel and Beauregard sweetpotatoes were collected in May of 1989 and 1990 from beds of sprouted storage roots that were apparently free of both bacterial stem and root rot and Fusarium root rot. The cut ends were dipped in inoculum as described above. Each treatment was replicated five times in 10-plant plots in a randomized complete block design. Cuttings were planted 30 cm apart with 90 cm between plots and 120 cm between rows. Before planting, 8-24-24 (NPK) fertilizer was incorporated into the soil at 400 lb/acre (448 kg/ha). The sweetpotatoes were grown according to recommended practices. Before September of each year, stems of five plants in 1989 and of all 10 plants in 1990 were split longitudinally at or near the soil line, and the incidence of stems with rotted tissue or black streaks in the vascular ring was recorded. At harvest in 1990, the total number of surviving plants per plot was recorded, and storage roots were counted and weighed.

Storage root inoculations. A micropipet tip containing 50 µl of inoculum or SDW was implanted about 1 cm deep into the median of each of 10 roots of Beauregard and Jewel. Inoculum consisted of a suspension of *E. chrysanthemi* (10^8 cfu/ml), microconidia and macroconidia of *F. solani* (10^6/ml), or combined inocula. Inoculated roots were placed in plastic vegetable baskets that were stacked and covered for 6 days with a black polyethylene bag left open at the bottom. The bag was then removed, and the roots were incubated at 25 ± 2 C for 5 wk. Roots were then cut crosswise

Present address of first author: Faculdade de Agronomia, UFRGS, Caixa Postal 776, Porto Alegre, RS, 90001, Brazil.

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through the point of inoculation, and diameter and depth of lesions were measured (5). This test was conducted twice.

Statistical analysis. For each experiment, inoculations with *E. chrysanthemi* or *F. solani* were tested as main effects, and the interaction between the two was determined by analysis of variance in SAS (SAS Institute, Cary, NC). When there was a significant difference between runs of the same test, each run was analyzed separately. In most experiments, the cultivars differed significantly or the interaction between cultivar and another variable was significant, and data for each cultivar were analyzed separately.

RESULTS

Stem inoculations. In greenhouse test 1, little disease was observed on Jewel plants inoculated with *F. solani* alone. Stem rot lesions on plants inoculated with *E. chrysanthemi* and *F. solani* combined were 111% longer than lesions on plants inoculated with *E. chrysanthemi* alone (Table 1). Lesion lengths on Jewel were similar in both tests (Table 1). On Beauregard plants in test 2, the main effect for inoculation with *E. chrysanthemi* was significant, but the interaction between pathogens was not significant (Table 1). Jewel plants inoculated with *E. chrysanthemi* alone or with both pathogens had lower dry weights than the noninoculated water control plants in test 1, but there was no significant interaction between pathogens (Table 1). Dry weight of plants in test 2 did not differ significantly among treatments.

Field experiments. The number of Beauregard plants with stem rot symptoms (either rotted tissue or black streaks in the vascular ring) was greatest in both years when plants were inoculated with both *E. chrysanthemi* and *F. solani* (Table 2). However, the results were significant ($P \leq 0.05$) only for inoculation with *E. chrysanthemi;* the effect of *F. solani* and the interaction of *E. chrysanthemi* and *F. solani* were not statistically significant (Table 2). Inoculation of Beauregard plants with *E. chrysanthemi* reduced yield in 1990 but increased the number of roots in 1989 (Table 3). Otherwise, yield and number of storage roots were not significantly different for any treatment in either year.

Storage root inoculations. Lesions in roots coinoculated with *E. chrysanthemi* and *F. solani* were not significantly larger than those in roots inoculated with *E. chrysanthemi* alone (Table 4). Lesions produced by *F. solani* were small and not significantly greater than the necrosis that occurred at the point of wounding in control roots. In most cases, lesions developed symmetrically around the end of the micropipet tips. In the second test, however, lesions that resulted from the combined inocula developed more extensively in the cortex of the root than around the micropipet tip in the central core of the root. The difference in external lesion diameter between the combined inoculation and the *E. chrysanthemi* inoculation was greater than the difference in internal lesion diameter, but the interaction between pathogens was not significant (Table 4).

DISCUSSION

A synergistic interaction between *E. chrysanthemi* and *F. solani* produced longer stem rot lesions in one greenhouse experiment with Jewel but not with Beauregard sweetpotato. In field experiments in 1989 and 1990, vascular symptoms were observed in inoculated plants, but differences in disease incidence were significant only for Beauregard plants inoculated in 1990 with *E. chrysanthemi.* Researchers have encountered difficulty in reproducing stem rot symptoms associated with either *E. chrysanthemi* or *F. solani* under field conditions. The higher temperatures in the greenhouse may have been more conducive to stem rot development than were field conditions in 1989 and 1990.

Overall, neither pathogen, alone or in combination, consistently affected yield. In fact, in 1989, inoculated Beauregard plants produced more storage roots than the controls. A moderate stress may actually induce sweetpotatoes to initiate formation of storage roots (3). Limited infection by *E. chrysanthemi* and/or *F. solani* under some environmental conditions may induce enough stress to stimulate storage root formation without stressing the plants so much that storage roots are smaller than in healthy plants. On the other hand, there was no evidence of decay in storage roots at or after harvest, and the severity of stem rot was less pronounced than has been observed on occasion to occur naturally. Environmental stress that did not occur during this study, such as heat, drought, or hypoxia, may be necessary for the initiation of root rot (8).

Resistance to *F. solani* and *E. chrysanthemi* in sweetpotato is expressed as restriction of lesion development at some time after inoculation (4,5). Results of

Table 1. Stem rot development on and dry weight of plants of sweetpotato cultivars Jewel and Beauregard 6 wk after inoculation with *Erwinia chrysanthemi* (Ech) and/or *Fusarium solani* (Fs) under greenhouse conditions

	Stem lesion length[a] (mm)			Dry weight of plants[a,b] (g)		
	Test 1,	Test 2		Test 1,	Test 2	
Treatment	Jewel	Jewel	Beauregard	Jewel	Jewel	Beauregard
Control	0.0	0.8	0.8	18.1	33.6	32.6
Ech	42.4	28.4	34.6	12.3	32.1	34.6
Fs	2.0	12.6	6.6	14.7	25.2	30.0
Ech + Fs	89.7	85.1	53.4	10.1	30.6	27.5
Source	$P > F$ from analysis of variance[c]					
Cultivar		0.2424			0.7068	
Ech	**0.0001***	0.0004*	0.0003*	0.0014*	0.6015	0.9236
Fs	**0.0182***	0.0062*	0.1584	0.0673	0.2003	0.0878
Ech × Fs	**0.0275***	0.0507	0.4434	0.3871	0.3711	0.3956
Block	**0.2611**	0.1868	0.4494	0.1858	0.6922	0.5321

[a] Means of five pots with five plants each.
[b] Plants were dried for 48 hr at 60 C.
[c] An asterisk indicates that the parameter was significant at $P < 0.05$.

Table 2. Disease incidence at harvest in field plots of Jewel and Beauregard sweetpotatoes inoculated with *Erwinia chrysanthemi* (Ech) and/or *Fusarium solani* (Fs) at transplanting

	Plants per plot with stem rot symptoms[a]			
	1989		1990	
Treatment	Jewel	Beauregard	Jewel	Beauregard
Control	0.0	0.4	0.0	0.0
Ech	1.0	1.0	0.0	0.5
Fs	0.8	0.8	0.0	0.2
Ech + Fs	1.4	3.3	0.2	1.0
Source	$P > F$ from analysis of variance[b]			
Cultivar	0.3913		0.0086*	
Ech	0.1391	0.0525	0.3370	0.0152*
Fs	0.2577	0.1343	0.3370	0.0838
Ech × Fs	0.6990	0.2152	0.3370	0.3644
Block	0.4830	0.6244	0.4449	0.2790

[a] Means of five plots with 10 plants each. Five plants per plot in 1989 and all 10 plants in 1990 were sampled. Stem rot symptoms included either soft rot or vascular discoloration.
[b] An asterisk indicates a parameter for which differences were significant at $P < 0.05$.

Table 3. Yield of Jewel and Beauregard sweetpotatoes inoculated with *Erwinia chrysanthemi* (Ech) and/or *Fusarium solani* (Fs) at transplanting

	Weight[a] (kg/plot)				Number of roots per plot[a]			
	1989		1990		1989		1990	
Treatment	Jewel	Beauregard	Jewel	Beauregard	Jewel	Beauregard	Jewel	Beauregard
Control	3.6	5.6	8.7	13.1	33	40	36	38
Ech	3.2	6.1	6.1	9.8	38	54	23	37
Fs	4.0	5.1	8.1	13.6	39	49	25	44
Ech + Fs	3.2	7.4	6.6	10.7	34	74	28	36
Source	$P > F$ **from analysis of variance**[b]							
Cultivar	0.0099*		0.0001*		0.0001*		0.0006*	
Ech	0.2812	0.0907	0.1648	0.0313*	0.9645	0.0072*	0.1776	0.2091
Fs	0.6780	0.5824	0.9480	0.6127	0.8587	0.0242*	0.3585	0.5275
Ech × Fs	0.6912	0.2524	0.6820	0.8528	0.2779	0.3715	0.0426*	0.3803
Block	0.0875	0.3478	0.4858	0.7719	0.0492*	0.2000	0.1897	0.6984

[a] Means of five plots with 10 plants each.
[b] Asterisks indicate parameters for which differences were significant at $P < 0.05$.

Table 4. Size of lesions in storage roots of Beauregard and Jewel sweetpotatoes inoculated with *Erwinia chrysanthemi* (Ech) and/or *Fusarium solani* (Fs)

	Internal lesion dimension[b] (mm)				
	Test 1		Test 2		External diameter (mm),
Inoculum[a]	Jewel	Beauregard	Jewel	Beauregard	test 2, Jewel[c]
None	0.4	1.9	0.0	0.2	0.0
Ech	9.7	22.4	14.0	43.0	12.0
Fs	5.1	7.3	8.3	7.6	7.5
Ech + Fs	9.8	30.9	17.1	41.7	26.5
Source	$P > F$ **from analysis of variance**[d]				
Cultivar	0.0016*		0.0001*		
Ech	0.0312*	0.0001*	0.0010*	0.0001*	0.0005*
Fs	0.3969	0.1900	0.0746	0.6192	0.0097*
Ech × Fs	0.4158	0.8302	0.4151	0.4722	0.3836
Block	0.7881	0.2237	0.8632	0.7402	0.8329

[a] A micropipet tip containing 50 µl of a suspension of *E. chrysanthemi* (10^8 cfu/ml) or *F. solani* (10^6 microconidia and macroconidia per milliliter) or both was implanted in roots.
[b] Roots were cut crosswise through the point of inoculation, and lesion diameter and lesion depth were measured at the point of inoculation. The internal lesion dimension is the mean of these two measurements.
[c] In the second test, lesions on Jewel were asymmetric, developing to a greater extent in the cortex than immediately around the micropipet tip.
[d] Asterisks indicate parameters for which differences were significant at $P < 0.05$.

the greenhouse experiments suggest that the sweetpotato plant may be less able to restrict development of both pathogens together than it is development of either pathogen alone. This may result in a prolonged infection and eventually lead to larger lesions. This reasoning also suggests that although an intermediate level of resistance to stem rot caused by either pathogen, such as exhibited by Jewel, may be sufficient to control either pathogen alone, it may not be sufficient when both pathogens occur together under more stressful conditions.

Two hypotheses have been suggested to explain the role of fungal pathogens in the development of potato blackleg: first, the increase in blackleg could be considered a function of the increased rate of seed piece decay, wherein the infection destroys the seed pieces and the newly developing stems; and second, systemic activity of the fungal pathogens present in the system could aid active or passive transport of the bacteria from the rotting seed piece into the stem (15). These hypotheses were considered valid for dry-rot fungi such as *F. solani* but not for wilt pathogens (15). In the case of postplant inoculations of potato with *F. solani,* colonization of the roots and vascular system by *F. solani* was considered to generally weaken the plant, making it more susceptible to the soft rot bacteria already established in the seed piece (15). On sweetpotato, although the bacteria can penetrate and enter the plant through wounded tissue on stem cuttings, the fungus also may facilitate penetration of *E. chrysanthemi* through the tissue as the fungal hyphae penetrate host cell walls.

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