Fusarium Wilt Race 1 on Lettuce

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Abstract. Three races of *Fusarium oxysporum* f.sp. *lactucae*, cause of fusarium wilt of lettuce, are known in Japan, where the pathogen was first observed in 1955. *Fusarium* wilt first affected commercial U.S. lettuce production in 1990 in Huron, Calif., but did not become a serious problem in the U.S. until 2001 when it reappeared in Huron and appeared in the Yuma, Arizona lettuce production area. Reactions of three fusarium wilt differentials ('Patriot', susceptible to races 1, 2 and 3; 'Costa Rica No. 4', resistant to race 1, and susceptible to races 2 and 3; and 'Banchu Red Fire', susceptible to races 1 and 3, and resistant to race 2) in a naturally-infected commercial field test and artificially-inoculated greenhouse tests, indicated presence of race 1 in the Yuma lettuce production area. Reactions of these differentials to an isolate from Huron confirmed the presence of race 1 in that area. Consistent with previous results from the U.S. and Japan, 'Salinas' and 'Salinas 88' were resistant to the Yuma and Huron isolates of race 1, whereas 'Vanguard' was highly susceptible. Limited F_1 and F_2 data indicate that resistance to race 1 in 'Costa Rica No. 4' and 'Salinas' is recessive. 'Calmar' is the likely source of resistance in 'Salinas' and 'Salinas 88'.

Fusarium wilt was first observed on lettuce (*Lactuca sativa* L.) in Japan in 1955 (Matuo and Motohashi, 1967), and since that time, three races of the pathogen *Fusarium oxysporum* f. sp. *lactucae* (*F. o. lactucae*) have been identified on lettuce in Japan (Fujinaga et al., 2001; Fujinaga et al., 2003). *Fusarium* was reported on lettuce in California in 1990, identified as *Fusarium oxysporum* f.sp. *lactucum*, and referred to as fusarium wilt (Hubbard and Gerik, 1993). The 1990 California pathogen was recently determined to be race 1 (Fujinaga et al., 2003). *Fusarium* wilt did not become a serious threat to lettuce production in the U.S. until 2001 when it first caused significant losses in the winter lettuce-growing district of Yuma, Arizona (Matheron and Koike, 2003), and re-occurred in the Huron lettuce district of California (Platts, unpublished). The pathogen was identified in Pajaro, Calif., in 2002 (S. Koike, University California Cooperative Extension, unpublished), which is contiguous

to the Salinas Valley where the U.S. summer lettuce production is centered.

Our objective was to identify the race of fusarium wilt present in the Yuma area and confirm the race identity of the California isolate. This information is necessary to initiate a breeding program. Toward that end we evaluated a limited number of F_1 individuals and an F_2 family to get a preliminary indication of the inheritance of resistance to fusarium wilt in lettuce adapted to Arizona and California growing conditions.

Materials and Methods

Plant materials. Seeds of the three fusarium wilt race differentials (Fujinaga et al., 2003) and race 1-resistant 'River Green' were provided by Sakata Seed America, Inc., Salinas, Calif. Their reported reactions to *F. o. lactucae* are as follows. 'Patriot' is susceptible to races 1, 2, and 3. 'Costa Rica No. 4' is resistant to race 1 and susceptible to races 2 and 3. 'Banchu Red Fire' is resistant to race 2 and susceptible to races 1 and 3. Seeds of 'Autumn Gold' (Ryder et al., 1991), 'Calmar' (Welch et al., 1965), 'Climax' (Thompson and Ryder, 1961), 'Empire' (ASHS, 1960), 'Salinas' (Ryder, 1979a), 'Salinas 88' (Ryder and Robinson, 1991), 'Vanguard' (Thompson and Ryder, 1961), 'Vanguard 75' (Ryder, 1979b), breeding line 8830 (Ryder, 1979a), and PI 251245 were produced by the USDA-ARS (J.D. McCreight). Conventional hybridization

procedures for lettuce (Ryder, 1999) were used to produce the F_1 and F_2 families used in this research.

Greenhouse tests. The first four tests reported here evaluated the reactions of the race differentials, 'Rivergreen', and seven other cultivars (Table 1). A fifth test evaluated the reactions of 22 F_1 individuals from 14 crosses of 'Vanguard' and 'Vanguard 75' with 'Costa Rica No. 4', 'Salinas', and 'Salinas 88' (Table 2). A sixth test evaluated the reactions of the parents and 25 F_2 individuals from a 'Vanguard' × 'Salinas' cross (Table 3). 'Calmar' and lettuce breeding line 8830 were compared with 'Salinas', 'Salinas 88' and 'Vanguard' in a final test (Table 4).

Lettuce seeds were sown in washed sand in plastic pots (10 × 10 × 10 cm deep). Seedlings were removed from the pots, inoculated as described below, and individually transplanted into washed sand in plastic pots (10 × 10 × 10 cm deep; one seedling per pot) on a greenhouse bench. Each test included 10 to 20 inoculated plants of each test entry along with 5 to 10 noninoculated controls and equal numbers of mock-inoculated (water) control plants. Plants were watered daily (greenhouse) with dilute (1:100) 20N–20P–20K fertilizer solution. No pesticides were applied to the plants in the greenhouse tests.

Fusarium isolate Fol121, originally isolated from lettuce in Yuma was provided by K.V. Subbarao, University of California, Davis. Inoculum consisted of a suspension of 5×10^6 conidia/mL prepared from Fol121 cultures grown on potato dextrose agar (PDA), as described by Hubbard and Gerik (1993). Roots of seedlings (about 14-d-old) were placed in the inoculum for 15 min (minimum) when transplanted as previously described. Seedlings were inoculated in random order, and, after transplanting, arranged in randomized blocks.

Foliar symptoms of inoculated and control plants in the greenhouse tests were evaluated 28 and 67 (test of F_1 individuals) d postinoculation (DPI) using a four-class scale, where 1 = no apparent disease, 2 = slight-moderate stunting, 3 = severe stunting and yellowing, and 4 = senesced (Hubbard and Gerik, 1993). Data were analyzed using the one-way (Fit Y by X) analysis of JMP (SAS Institute, Inc.). Plants that were rated 1 were classified resistant; those rated 2, 3, or 4 were classified susceptible.

Field tests. Seeds of the fusarium wilt differentials 'Patriot' and 'Costa Rica No. 4', and 'Vanguard' and 'Salinas' were planted in a commercial lettuce field near Yuma on 6 December 2002 (water date was 6 Dec. 2002). Each experimental plot was 15 m in length and consisted of two rows about 0.3 m apart on conventional lettuce beds spaced on 1 m centers. There were four randomized blocks. The experimental plots were grown following standard commercial practices for the desert southwest U.S. (Whitaker et al., 1974). The same disease rating scale was used in the field test, but class 2 was not discernible. Data were analyzed using the two-way analysis (Fit Model) of JMP (SAS Institute, Inc.). Plants that were rated 1 were classified resistant; those rated 3 or 4 were classified susceptible.

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Root infection was verified in greenhouse and field tests by examining cut roots for red or brown discoloration characteristic of infection by *F. o. lactucae* (Hubbard and Gerik, 1993; Matuo and Motohashi, 1967). To confirm that *F. o. lactucae* was the cause of the observed symptoms, selected roots were cultured on PDA and the pathogen was identified based on morphological characteristics as described by Hubbard and Gerik (1993).

Results and Discussion

There were significant differences between entries included in the four greenhouse tests reported herein (Table 1). All controls (noninoculated and mock-inoculated) in all greenhouse tests were asymptomatic (data not shown). 'Patriot' was susceptible to the Yuma isolate, but was not included in the test with the Huron isolate (Table 1); its mean disease reaction ranged from 1.5 to 3.1. 'Costa Rica No. 4' was resistant to the Yuma and Huron isolates; its means ranged from 1.0 to 1.2 (Table 1). 'Banchu Red Fire' was not infected by the Yuma isolate in greenhouse test 1, but was susceptible to it in greenhouse test 3 in which the mean disease reaction of 'Patriot' was 3.1 (Table 1). These data are consistent with results from Japan which showed 'Patriot' susceptible to races 1, 2, and 3; 'Costa Rica No. 4' resistant to race 1 and susceptible to races 2 and 3; and 'Banchu Red Fire' resistant to race 2 and susceptible to races 1 and 3.

'Vanguard' was susceptible; its mean disease ratings ranged from 2.9 to 3.3 (Table 1). 'Vanguard 75' was susceptible in the only test in which it was included (Table 1). These results were consistent with previous results in response to inoculation with one of the original Huron isolates (Hubbard and Gerik, 1993).

'River Green' was resistant (Table 1). Resistance reactions of 'Salinas' and 'Salinas 88' in these tests were consistent with previous results when inoculated with Huron isolates (Hubbard and Gerik, 1993) and a Japanese isolate of race 1 (Tsuchiya and Hideki, 2002). The intermediate reactions of 'Empire' and 'Autumn Gold' were consistent with previous results in response to inoculation with one of the original Huron isolates (Hubbard and Gerik, 1993) (Table 1).

In the field test, 'Patriot' exhibited a moderate level of disease and was not significantly different from 'Climax' (Table 1). 'Costa Rica No. 4' and 'River Green' were resistant and did not exhibit any foliar symptoms.

Of 22 inoculated F_1 individuals from 14 'Vanguard' crosses, 11 were diseased 28 DPI; an additional five individuals were symptomatic 46 DPI (Table 2). In the test of the F_2 'Vanguard' $\times$ 'Salinas 88' family, 'Salinas' was resistant, 'Vanguard' was susceptible, and the F_2 segregated in a close fit to a 1 resistant (disease class 1):3 susceptible (disease classes 2 + 3 + 4) ratio, $\chi^2 = 0.60$ with Yates correction (Yates, 1931), $P = 0.45$ (Table 3).

These results demonstrated the presence of *F. o. lactucae* race 1 in Yuma and confirmed the race in Huron to be race 1 (Fujinaga et al., 2003). 'Salinas', 'Salinas 88', and 'River

Green' were clearly resistant in the greenhouse, but data from naturally infected field tests planted during the Empire planting slot (1 to 20 Sept.), a period when ambient temperatures are higher than in the December field test, are needed to better ascertain their value for development of resistant cultivars adapted to the Yuma production area when disease pressure is especially severe (Matheron and Koike, 2003). Temperature effects have been shown to influence the reactions of 'Salinas 88' and 'Patriot' in response to race 1 in Japan; disease severity increased as the mean daily temperature increased (Tsuchiya and Hideki, 2002).

The F_1 data clearly indicate that resistance in 'Costa Rica No. 4', 'Salinas', and 'Salinas 88' to race 1 is recessively controlled. The limited F_2 data suggest simple inheritance, but need verification. This is the first report of data on the inheritance of resistance to fusarium wilt in lettuce. Responses of line 8830 and 'Calmar' (Table 4), the parents of 'Salinas' (Ryder, 1979a), suggest that the resistance in 'Salinas' may have come from 'Calmar' (Welch et al., 1965). The pedigree of 'Vanguard' has the same origin as 'Salinas' but does not include 'Calmar' (Ryder, 1979a; Thompson and Ryder, 1961). 'Salinas 88' originated from two crosses

Table 1. Mean disease ratings and disease reactions of three lettuce fusarium wilt race differentials and seven other lettuce cultivars to inoculation in four greenhouse tests and natural infection in a field test in Yuma.

Entry	Greenhouse tests ^z				Yuma field	Disease reaction
	1	2	3	4		
Fusarium race differentials						
Patriot	1.5	2.4	3.1	---	2.4	S
Costa Rica No. 4	1.0	1.1	1.2	1.0	1.0	R
Banchu Red Fire	1.0	---	2.7	1.7	---	S
Other lettuce cultivars						
Autumn Gold	1.5	---	---	---	---	---
Climax	3.3	---	---	---	2.2	S
Empire	1.4	---	---	---	---	R
River Green	1.2	---	---	---	1.0	R
Salinas	---	---	1.6	1.0	---	R
Salinas 88	1.2	1.4	---	---	---	R
Vanguard	3.1	2.9	3.3	2.3	---	S
Vanguard 75	---	3.0	---	---	---	S
LSD _{0.01}	0.8	1.1	1.1	0.7	0.8	
Number per entry	10	10	10	10	>140	

^aFour greenhouse tests: tests 1, 2, and 3 using an isolate from Yuma, Ariz., and test 4 using an isolate from Huron, Calif. All noninoculated and mock-inoculated (water) controls were asymptomatic.

Table 2. Reaction of putative F_1 plants from crosses of susceptible 'Vanguard' with resistant 'Costa Rica No. 4', 'Salinas', and 'Salinas 88' to inoculation with *Fusarium oxysporum* f.sp. *lactucae* Yuma isolate in a greenhouse; numbers of diseased plants at 28 and 67 d postinoculation (DPI), and total number of inoculated plants.

Cross	No. of crosses	Diseased plants ^a		Total inoculated plants
		28 DPI	67 DPI	
Vanguard $\times$ Costa Rica No. 4	4	3	4	6
Vanguard 75 $\times$ Costa Rica No. 4	2	2	2	4
Vanguard $\times$ Salinas	2	2	3	3
Vanguard $\times$ Salinas 88	3	1	3	5
Vanguard 75 $\times$ Salinas	3	3	4	4

^aAll noninoculated and mock-inoculated (water) controls were asymptomatic.

Table 3. Reaction of an F_2 family from a cross of 'Vanguard' with 'Salinas 88' and its parental lines to inoculation with *Fusarium oxysporum* f.sp. *lactucae* Yuma isolate in a greenhouse; 28 d postinoculation.^a

Entry	Mean ^b	Disease class			
		1	2	3	4
Vanguard	2.8 a	1	2	5	2
Salinas	1.2 b	9	0	1	0
F_2 Vanguard $\times$ Salinas	1.9 b	9	11	7	0

^aAll noninoculated and mock-inoculated (water) controls were asymptomatic.

^bMeans followed by different letter are significantly different at $P_{0.01}$.

Table 4. Reactions of 'Salinas', 'Salinas 88', 'Vanguard' and two of their progenitors to inoculation with *Fusarium oxysporum* f.sp. *lactucae* Yuma isolate in a greenhouse; 28 d postinoculation.^a

Entry	Mean ^b	Disease class			
		1	2	3	4
Salinas	1.5 a	15	1	3	1
Salinas 88	1.5 a	15	2	1	2
Vanguard	2.8 a	7	1	1	11
Calmar	1.9 ab	13	0	3	4
8830	2.4 bc	6	5	4	5

^aAll noninoculated and mock-inoculated (water) controls were asymptomatic.

^bMeans followed by different letter are significantly different at $P_{0.01}$.

that involved ‘Vanguard’, and outcrosses to PI 251245, ‘Calmar’, and ‘Salinas’ before concluding with a series of three backcrosses to ‘Salinas’ (Ryder and Robinson, 1991).

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