Field Resistance of California Strawberries to *Verticillium dahliae* at Three Conidial Inoculum Concentrations

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Abstract. Runner plants from strawberry (*Fragaria × ananassa* Duch.) genotypes previously identified as relatively susceptible, intermediate, and resistant to wilt caused by *Verticillium dahliae* Kieb. were inoculated with a conidial suspension containing a mixture of five isolates of *V. dahliae* at 10⁴, 10⁵, and 10⁶ conidia/mL. Disease symptoms were scored as the number of dead or severely stunted plants per plot, or on the basis of a subjective phenotypic resistance score assigned to each plot on eight dates during the spring after planting. Overall disease symptoms increased with inoculum concentration; resistance scores for all genotypes were 4.8, 3.7, and 3.2, and the percentages of plants stunted or dead were 6.8, 32.6, and 44.9 for the three conidial concentrations, respectively. The relative resistance categories were separable at concentrations of 10⁶ and 10⁵, whereas no separation was obtained at 10⁴ conidia/mL. Genotypes originally classified as intermediate in resistance performed more like susceptible types at the highest conidial concentration. Significant resistance category × conidial concentration level interactions were detected for resistance score but not for the number of dead or severely stunted plants per plot. Regression coefficients for resistance score and percentage of stunting on conidial concentrations were statistically significant only for susceptible and intermediate genotypes. Some stunting was detected within all resistance categories at the highest conidial concentration, and the practical value of the resistance developed to date will depend ultimately on realistic field inoculum levels.

Prior to the widespread use of soil fumigants, *Verticillium dahliae* was considered among the most important pathogens for commercial strawberry production in California (Thomas, 1932). This disease continues to be a major concern where strawberries are managed in perennial planting systems (Maas et al., 1989; Paulus, 1990; Wilhelm et al., 1961; Wilhelm and Paulus, 1980). *Verticillium* wilt is controllable by preplant soil fumigation, but the soil population is only reduced and not eradicated by each soil treatment; repeated fumigation treatments may be needed to reduce infection to acceptably low levels (Wilhelm et al., 1961).

Breeding for resistance may be an alternative for controlling *V. dahliae* damage in strawberry, as there is substantial genetic variation for resistance to this disease in many breeding populations (Bringhurst et al., 1966, 1968; Maas et al., 1989; Shaw et al., 1996). However, despite substantial screening efforts, only one *F. × ananassa* genotype highly resistant to *V. dahliae* has been reported (Maas et al., 1989), suggesting that such genotypes will be rare in most breeding populations (Shaw et al., 1996). Genotypes with field resistance ex-

ceeding that developed to date will be required if cultivars are expected to tolerate field disease pressures. Also, resistance of strawberry to *V. dahliae* is polygenic in inheritance (Bringhurst et al., 1968; Shaw et al., 1996) and symptom development may vary with inoculum concentration (Gordon et al., 1989; Harris and Yang, 1996; Resende et al., 1995). Our objective was to 1) determine the consistency of field resistance to wilt caused by *V. dahliae* at three conidial inoculum concentrations and 2) evaluate the consequences of any genetic × inoculum concentration interactions detected for obtaining genetic progress in resistance breeding programs.

Materials and Methods

Twelve genotypes from the Univ. of California (UC) strawberry breeding population, four each identified as relatively resistant, intermediate, or susceptible to *V. dahliae* in a previous study (Shaw et al., 1996), were evaluated using replicated plots of runners (Table 1). These resistance categories were established using disease symptom scores obtained previously and sets of genotypes were generated with scores of 1.0 to 1.6 for susceptible, 2.0 to 2.4 for intermediate, and 3.1 to 4.5 for resistant types, with a possible range of 1 to 5. Because the genetic variation for this resistance appears polygenic and continuous, this discrete categorization is somewhat arbitrary. These sets of genotypes were chosen to represent the range of genetic alternatives within the UC program. For reference, genotypes in

the most resistant category were equivalent in expression of disease symptoms to the most resistant cultivars from other North American breeding programs in the original trial. However, the stability of this result across production environments and inoculum sources is uncertain.

Field plots were prepared for planting and treated with 2 methyl bromide : 1 trichloronitromethane (chloropicrin) at 392 kg·ha⁻¹ at the Wolfskill Experimental Orchard near Davis, Calif. Runner plants from each genotype were harvested on 28 Sept., trimmed to retain two leaves, and stored at 2 °C until planting on 4 Oct. (Shaw, 1993). Before planting, all runners were dipped for 5 min in a solution containing a five-isolate mix of *V. dahliae* at ≈10⁴, 10⁵, or 10⁶ conidia/mL, according to previously described procedures (Shaw et al., 1996). Isolates of *V. dahliae* were originally obtained from symptomatic plants in production fields near Watsonville, Calif. Thereafter, plants were treated according to recommendations for commercial winter plantings (Welch, 1989).

Each genotype/conidial concentration combination was replicated twice, and was represented by a single plot of five inoculated runners in each of two blocks. Two non-inoculated runners of each genotype were planted at the end of each inoculated plot, with 60 cm between the last inoculated plant and the noninoculated control. All evaluations were made relative to the noninoculated standards.

Symptoms were scored as the number of dead or severely stunted plants per plot or on the basis of a subjective phenotypic resistance score assigned to each plot. As in the previous trial (Shaw et al., 1996), the subjective resistance score was assigned as an integer from 1 to 5, with a lower score indicating more severe disease symptoms. Evaluations of disease symptoms were performed on eight dates during the spring after planting: 5 Apr., 27 Apr., 3 May, 12 May, 17 May, 9 June, 22 June, and 7 July. Two observers performed these evaluations, with half of the evaluation dates contributed by each. Further inferences about genetic resistance were made using the average values for each of the two disease symptom variables over all evaluation dates. The number of stunted or dead plants was converted to percentages and transformed using

Table 1. Resistance scores² for genotypes used to evaluate the relationship between resistance category and inoculum concentration.

Resistance category	Genotype	Resistance score
Susceptible	Seascape	1.0
	Cal 88.31-3	1.1
	Chandler	1.3
	Cal 89.218-31	1.6
Intermediate	Camarosa	2.0
	Anaheim	2.3
	Carlsbad	2.4
	Selva	2.4
Resistant	Sunset	3.1
	Cal 90.241-2	3.6
	Cal 90.277-4	3.7
	Laguna	4.5

²Values obtained from Shaw et al. (1996).

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Table 2. Means and standard deviations (in parentheses) for subjective resistance scores^a and percentage of plants severely stunted or dead due to inoculation of strawberry genotypes from three resistance categories with three conidial concentrations.

Resistance category	Disease symptom variable					
	Conidial concn (no./mL)					
	10 ⁴		10 ⁵		10 ⁶	
	Score	Stunted or dead (%)	Score	Stunted or dead (%)	Score	Stunted or dead (%)
Susceptible	4.73 (0.33)	11.1 (5.9)	3.00 (1.35)	48.9 (33.4)	2.09 (0.63)	68.2 (18.6)
Intermediate	4.93 (0.10)	4.3 (5.1)	3.68 (0.66)	31.8 (19.0)	2.96 (1.00)	48.9 (18.3)
Resistant	4.88 (0.15)	5.0 (3.6)	4.55 (0.41)	18.2 (13.1)	4.61 (0.64)	17.5 (20.9)

^aSubjective resistance scores were assigned as an integer from 1 to 5, with a lower score indicating more severe disease symptoms (Shaw et al., 1996).

Table 3. Analysis of variance results for variables scored to describe resistance to *Verticillium dahliae* in susceptible, intermediate, and resistant strawberry genotypes evaluated using three concentrations of conidial inoculum.

Source	df	Disease symptom variable	
		Stunted or dead (%)	Resistance score
Block	1	0.01	0.55
Conidial concentration (C)	2	1.67**	17.83**
Resistance category (R)	2	0.85**	13.04**
C × R	4	0.18*	2.55**
Within cross	62	0.08	0.60

**Borderline significance ($P = 0.08$) or significant at $P < 0.01$, respectively.

arcsin-square roots prior to statistical analyses.

Analyses of variance (ANOVAs) were conducted for each disease symptom variable, treating blocks, resistance categories, and conidial concentrations as fixed effects using SAS procedure GLM (version 6.07) and Type III sums of squares (SAS Institute, Cary, N.C.). All interactions with blocks were pooled into a common error term. Interactions between resistance category and conidial concentration were further evaluated, first by conducting an analysis of simple main effects (Steel and Torrie, 1960), then by performing regression analyses of disease symptom variables for each resistance category on the logarithm of the conidial concentration.

Results and Discussion

Preliminary resolution of disease symptom variables suggested large differences among the plants treated with different conidial concentrations. Over all three resistance categories, resistance scores were 4.8 (± 0.06), 3.7 (± 0.24), and 3.2 (± 0.27), and the percentage of plants severely stunted or dead was 6.8 (± 1.6), 32.6 (± 5.7), and 44.9 (± 6.1) for 10⁴, 10⁵, and 10⁶ conidia/mL, respectively. The original resistance categories were easily distinguishable at 10⁵ and 10⁶ conidia/mL, but no clear separation was apparent at 10⁴ conidia/mL (Table 2). Also, genotypes originally classified as intermediate in resistance performed more like susceptible types at higher conidial concentrations.

ANOVAs detected highly significant differences among resistance categories and among conidial concentrations for both disease symptom variables (Table 3). However, resistance category × conidial concentration interactions were highly significant ($P < 0.01$)

for resistance score and approached significance ($P < 0.08$) for the number of dead or severely stunted plants per plot; thus, further resolution of these results was needed. Analysis of simple main effects demonstrated that interactions were the likely result of two causes. First, resistance categories were statistically indistinguishable at the lowest conidial concentration for either resistance score or the percentage of stunted or dead plants ($F = 0.35$ to 0.40, $P = 0.67$ to 0.70) but differed significantly for both symptom variables ($F = 3.38$ to 8.13, $P < 0.05$ and $F = 10.85$ to 21.91, $P < 0.001$) with intermediate and high conidial concentrations, respectively. Second, no significant differences among conidial concentra-

tion levels were detected for either disease symptom variable when evaluated using genotypes in the most resistant category ($F = 1.01$ to 1.10, $P = 0.34$ to 0.37), but were easily detected using intermediate ($F = 9.70$ to 13.25, $P < 0.001$) and susceptible ($F = 13.82$ to 24.17, $P < 0.001$) genotypes, respectively. These interactions can be visualized as differences in response to increasing inoculum concentrations for the three resistance categories (Fig. 1).

Further evaluation of these interactions using regression analyses verified the results of simple main effects analysis. Significant regression of resistance score and the percentage of stunted or dead plants was obtained on the logarithm of the conidial concentration for intermediate and susceptible types, and coefficients were similar in magnitude (Table 4). Conversely, no significant regression of disease symptom variables on conidial concentration was detected for the most resistant types.

These differing regression results suggest that only the most resistant genotypes are likely to survive high inoculum concentrations in the production field. Even here, non-significant levels of stunting and mortality (17.5%, $P < 0.09$) and submaximum compos-

Fig. 1. *Verticillium* resistance scores for relatively resistant, intermediate, and susceptible strawberry genotypes at three conidial concentrations. Subjective resistance scores were assigned as an integer from 1 to 5, with a lower score indicating more severe disease symptoms (Shaw et al., 1996). Inoculum of *V. dahliae* was a mixture of five isolates obtained from symptomatic strawberry plants near Watsonville, Calif.; conidial concentration was adjusted using a haemocytometer after 2 weeks' growth on acidified potato dextrose agar.

Table 4. Regression analyses results for variables scored to describe resistance to *Verticillium dahliae* in susceptible, intermediate, and resistant strawberry genotypes evaluated using three concentrations of conidial inoculum.

Resistance category	Source	df	Disease symptom variable	
			Stunted or dead (%)	Resistance score
Susceptible	Block	1	0.06	0.01
	Regression	1	2.23**	27.94**
	Residual	21	0.10	0.87
	b (±SE)		0.162 (0.035)	-0.574 (0.101)
Intermediate	Block	1	0.01	0.17
	Regression	1	1.57**	15.43**
	Residual	21	0.07	0.64
	b (±SE)		0.136 (0.028)	-0.427 (0.087)
Resistant	Block	1	0.14	0.87
	Regression	1	0.13	0.78
	Residual	21	0.07	0.31
	b (±SE)		0.039 (0.029)	-0.097 (0.60)

**Significant at $P < 0.01$.

ite scores (4.61, $P < 0.20$) were detected for the most resistant genotypes at the highest conidial concentration. Extrapolation beyond the range of these conidial concentrations and to field conditions where a diversity of inoculum types can be anticipated must be made with some caution (Maas et al., 1985). However, these results point to realistic concerns about viability of the most resistant UC genotypes we studied under field conditions with very high levels of inoculum.

These results underscore the need for estimation of field inoculum concentrations and for the establishment of realistic disease thresholds in designing resistance breeding programs. Further, the presence of disease symptoms at high inoculum concentrations, even for the most resistant genotypes now available, points to the importance of nongenetic methods for control of *V. dahliae* in strawberry. Cultivars with resistance equal to the highest expressed

here might be acceptable in concert with an integrated system that reduces inoculum levels. However, given the experience with this pathogen to date (Maas et al., 1989), it is unlikely that any level of *Verticillium* resistance yet identified will be sufficient without some additional control measure.

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