

Response to Family Selection for Field Resistance to *Verticillium dahliae* in California Strawberries

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ABSTRACT. Strawberry genotypes were retained from biparental progenies previously identified as either relatively susceptible or resistant to wilt caused by *Verticillium dahliae* based on a phenotypic resistance score. Runner plants from these selected genotypes were inoculated with a conidial suspension containing a mixture of five isolates obtained from symptomatic strawberry plants at 10^6 conidia/mL, then scored for disease symptoms. Genotypes from resistant progenies had significantly higher phenotypic resistance scores (1 = severe symptoms, 5 = no detectable symptoms) than those from susceptible progenies (4.15 vs. 2.23), and there also was a correlated selection response for the percent plants severely stunted or dead (26.4 and 69.1 for resistant and susceptible selections, respectively). Comparisons of the resistance scores for selected groups with those for the original parents (2.76) indicated that selection had changed relative resistance significantly in both directions and that realized response had been 24% to 43% larger than predicted for selection in both directions. Although several of the selections from resistant progenies were highly susceptible, five of the 21 resistant selections had resistance scores outside the range of the original parents, representing possible transgressive segregants. This comparison is limited by the precision with which individual resistance scores are estimated and by the scope of the disease symptoms in this trial. Detecting genotypes with sufficient resistance ultimately will depend on development of screening methods with greater sensitivity than those used here.

Plant collapse resulting from *Verticillium dahliae* Kreb. infection has been recognized as a major obstacle to strawberry production in California for several decades. Plantations established in infested soils often suffer 50% or greater mortality (Thomas, 1932; Wilhelm and Koch, 1956). The presence of genetic variation for resistance to verticillium wilt has been demonstrated in domestic and wild strawberry populations (Bringhurst et al., 1966, 1968; Maas et al., 1989) and, more recently, among genotypes within the current Univ. of California (UC) strawberry breeding population (Shaw et al., 1996). Resistance appears to be polygenic in its inheritance, conditioned by additive and dominance genetic effects, and genotypes with exceptional resistance are rare (Bringhurst et al., 1968; Maas et al., 1989; Shaw et al., 1996). Regardless of any potential genetic complications, heritabilities for resistance are high (Bringhurst et al., 1968; Shaw et al., 1996) and breeding for resistance is frequently cited as an alternative to preplant soil fumigation for mitigating the consequences of *V. dahliae* in strawberry (Maas, 1984; Watson et al., 1992).

The objectives of our experiments were 1) to determine the change in field resistance to wilt caused by *V. dahliae* from a single cycle of selection based on progeny (full-sib family) selection and 2) to compare realized response with that previously predicted for this breeding population (Shaw et al., 1996). Several methods are available for genetic improvement of polygenic pest resistance (Falconer, 1981; Shaw, 1996), each with their relative merits. Ultimately, our results can be used to evaluate the opportunities for further genetic advance via progeny selection, alone and in combination with other testing systems.

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Materials and Methods

Two experimental plots located at the Wolfskill Experimental Orchard near Davis, Calif., were prepared for planting and treated with 2 methyl bromide : 1 trichloronitromethane (chloropicrin) at 392 kg-ha^{-1} in 1993. Seedlings from 50 biparental progenies, generated by crosses among 23 cultivars and advanced selections from the UC strawberry breeding population (with one to six crosses/parent) were established in these plots: the first plot was dedicated to *V. dahliae* inoculation trials and the second to seedling performance trials. Seedling germination began on 17 June 1993; germinants were transplanted to peat-pots during the first week of August.

To obtain disease resistance ratings, 20 seedlings from each progeny were inoculated with a conidial suspension containing 10^6 conidia/mL of five *V. dahliae* isolates obtained from symptomatic strawberry plants near Watsonville, Calif. Seedlings were inoculated, transplanted to the field on 30 Sept. 1993, then evaluated for disease symptoms in Spring 1994 as described and reported previously (Shaw et al., 1996). Results from these 1994 seedling inoculation trials were used to estimate cross-mean heritabilities for a subjective phenotypic resistance score ($h_c^2 = 0.88 \pm 0.24$) and for the percent plants severely stunted or dead ($h_c^2 = 0.61 \pm 0.21$), to estimate the genetic correlation between these traits ($r_g = -0.958$), and to score the relative resistance of the 50 progenies.

In the seedling performance trial, 50 to 120 seedlings from each of the 50 biparental progenies were transplanted to the field on 23 Sept. 1993 and were evaluated in Spring 1994 for a number of subjective and objective performance characteristics with the intention of selecting genotypes with commercial potential. Altogether, 124 genotypes were retained from these 50 progenies for further performance testing, irrespective of their performance in disease screening trials, and a subset of these genotypes were used to form the selected populations in the *Verticillium* trial.

Twenty-one of the 124 seedlings chosen for other performance characteristics were retained from the five biparental progenies

with the highest resistance scores to form a selected population of genotypes resistant to *V. dahliae*, and selection for susceptible genotypes was conducted by retaining 12 selections of the original 124 from the six biparental progenies with the lowest scores. Although these sets of genotypes are not a random sample of the original population, the only selection pressure relevant to genetic resistance is that due to progeny-mean selection for resistance or susceptibility.

Selection response for the subjective resistance score was predicted as (Falconer, 1981):

$$R_c = i_c h_c^2 \sigma_c \quad [1]$$

In [1], i_c is the standardized selection differential among progeny means ($i_c = 1.8$ and -0.8 for resistant and susceptible selections, respectively), obtained as the average difference between standardized resistance scores for selected progenies and the population mean score, with selected population means calculated using the five or six progenies included in each selection treatment and individual progeny scores weighted by the number of genotypes per selected progeny; h_c^2 is the heritability appropriate to selection based on progeny means; and σ_c is the phenotypic standard deviation of progeny means for the selected trait and was estimated as 0.611 from Shaw et al. (1996). Correlated response to selection for the percent plants stunted or dead was predicted as (Falconer, 1981):

$$CR_c = i_c h_{c(s)} h_{c(p)} r_g \sigma_c \quad [2]$$

In [2], $h_{c(s)}$ and $h_{c(p)}$ are square roots of the progeny-mean heritabilities for the subjective score and the percent plants stunted or dead, and r_g is the genetic correlation between the two traits.

Genotypes retained from the most resistant and susceptible

Table 1. Means and standard deviations for subjective resistance scores (1 = severe symptoms, 5 = no detectable symptoms) and the percent plants severely stunted or dead for populations of strawberry genotypes selected for resistance or susceptibility to *Verticillium dahliae* and their parental population.²

Selection Category	N	Disease symptom variable			
		Resistance score		Stunted or dead (%)	
		\bar{X}	SD	\bar{X}	SD
Resistant	42	4.15	0.84	26.4	21.3
Susceptible	24	2.23	0.60	69.1	17.3
Parents	46	2.76	0.96	58.9	21.7

²Results for the 23 parents were obtained in 1994; individual-plot values were corrected for yearly mean differences using a sample of 21 genotypes common to trials conducted in both years prior to calculation of descriptive statistics.

Table 2. Mean squares from the analysis of variance of variables scored to describe resistance to *Verticillium dahliae* for susceptible and resistant genotypes.

Source	df	Disease symptom variable	
		Resistance score	Stunted or dead (%)
Block (B)	1	0.02	0.01
Resistance category (R)	1	57.55**	2.86**
B × R	1	0.72	0.03
Error	62	0.59	0.04

***Significant at $P < 0.05$ or 0.01 , respectively.

progenies were evaluated individually in 1995 on soil prepared for planting and treated with 2 methyl bromide: 1 chloropicrin at 392 kg-ha⁻¹ in August 1994. Runner plants from each genotype were harvested on 28 Sept. 1994, trimmed to retain two leaves, and stored at 2 °C until planting on 4 Oct. (Shaw, 1993). Before planting, all runner plants were dipped for 5 min in a suspension containing $\approx 10^6$ conidia/mL of the same five isolates used in progeny evaluations. Inoculations were performed and conidial suspensions were generated according to procedures described previously (Shaw et al., 1996). Thereafter, plants were treated according to recommendations for commercial winter plantings (Welch, 1989).

Each genotype was represented by two plots of five runners, one 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 non-inoculated control; all evaluations were made relative to the non-inoculated 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 (Shaw et al., 1996). The phenotypic resistance score was assigned as an integer from 1 to 5, with a the maximum value indicating no verticillium wilt symptoms, smaller numbers indicating greater wilting or stunting symptoms, and the minimum value indicating complete plant mortality. Symptoms progressed at differing rates depending on the relative susceptibility of specific genotypes (Shaw et al., 1996), and evaluations were performed on eight dates during the spring after planting (1995) to incorporate this differential into a composite score: 5 and 27 Apr.; 3, 12, and 17 May; 9 and 22 June; and 7 July. Two observers each performed four of the evaluations. Further inferences about genetic resistance were made using the average values for each of the two disease symptom variables over all observation dates.

Realized selection responses were calculated by comparing trait means for selected resistant or susceptible populations with those for the 23 parental genotypes obtained in 1994 with an identical inoculation trial (Shaw et al., 1996). Because scores differed across years, the 1994 results were adjusted to 1995 standards (years-mean correction) using 21 genotypes common to trials conducted in both years.

Results and Discussion

Large differences were detected between the bidirectionally selected populations for the resistance score and the percent plants stunted or dead (Table 1). Differences between the selected populations were highly significant ($P < 0.01$) for both resistance traits (Table 2). Indirect comparisons conducted after adjusting results for the 23 parents obtained in 1994 (Shaw et al., 1996) for yearly mean differences demonstrated significant differences between selected and parental populations for the phenotypic resistance score in both directions ($t = 2.81$ and $t = 7.37$, $P < 0.01$ for selection in the susceptible and resistant directions) and for the percent plants stunted or dead ($t = 2.15$, $P = 0.036$, and $t = 7.21$, $P < 0.01$).

Realized selection response for the resistance score was larger in absolute magnitude for the resistant population than for the susceptible population (Table 3). However, this can be attributed to larger absolute selection intensity applied to the resistant direction (1.80 vs. -0.80), and, in fact, realized response was somewhat larger than predicted for selection in both directions (Table 3). The failure to obtain similar selection differentials with similar proportions of progenies retained (5 or 6 of 50 for selection in resistant and susceptible directions, respectively) may indicate that allele fre-

quencies in the parental population are skewed toward susceptibility. Alternatively, it is likely that the most susceptible category includes a range of genotypes and that this asymmetry is an artifact of the scoring system and disease threshold invoked in this study.

Several of the genotypes from relatively resistant progenies were highly susceptible (Fig. 1), indicating that a two-stage process including clonal testing of seedlings from selected progenies will be needed if family selection is to be useful in identifying exceptionally resistant genotypes. Conversely, five of the 21 resistant selections had scores greater than that of the most resistant parent (4.50); whether these represent transgressive segregants will require greater precision in estimating individual genotypic values than that available in this study. In fact, several of the selections were free of disease symptoms and, therefore, received maximum scores. Further progress, and the capability to develop genotypes with resistance sufficient to withstand high disease pressures in production fields, might require a test that invokes a greater disease threshold than was obtained on our study.

Table 3. Predicted and realized selection response for subjective resistance scores and correlated selection response for the percent plants severely stunted or dead for populations of strawberry genotypes selected for resistance or susceptibility² to *Verticillium dahliae* Kieb.

Selection Category	Resistance score		Stunted or dead (%)	
	Predicted	Realized	Predicted	Realized
Resistant	0.97	1.39	25.3	33.0
Susceptible	0.43	0.53	7.3	10.0
Bidirectional	1.40	1.92	32.6	43.0

²Realized responses were calculated as differences from parental means after correction for yearly mean differences using a sample of 21 genotypes common to trials conducted in both years.

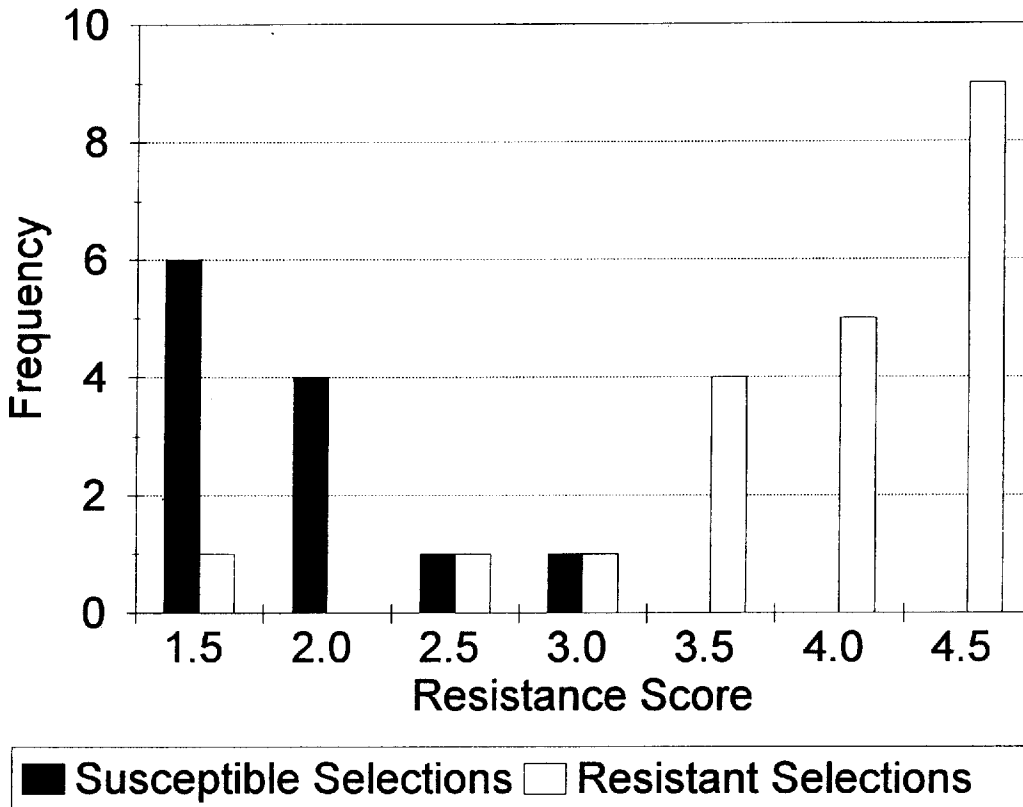


Fig. 1. Distribution of individual genotypes within populations selected for resistance or susceptibility to *Verticillium dahliae*.

Direct selection of resistance in inoculation trials may have some genetic advantages over selection of random individuals from resistant progenies, but choice of breeding strategy depends on several tradeoffs (Falconer, 1981). Selections obtained through screening of seedlings after inoculation might speed the rate of short term genetic progress because their individual genotypic effects are scored directly. Conversely, progeny heritabilities are larger than individual plant heritabilities and individual seedlings free of disease symptoms may be escapes, both factors that favor family selection. Individual seedling evaluations were somewhat less reliable than runner plant evaluations due to differences in the inoculation procedure (Shaw et al., 1996), so the benefits of scoring seedlings directly for resistance are uncertain. Also, individual selections obtained from direct-inoculation trials will be infected with disease and will require special handling. No single strategy is likely to be sufficient alone, and family selection followed by testing of runner-propagated clones is a relatively efficient compromise.

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