Inheritance of Powdery Mildew Resistance in Greenhouse-Grown Versus Field-Grown California Strawberry Progenies

M. D. Nelson, W. D. Gubler, and D. V. Shaw

First and second authors: Department of Plant Pathology; and third author, Department of Pomology, University of California, Davis 95616.

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ABSTRACT

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Seedlings from 17 strawberry (*Fragaria* × *ananassa*) progenies were evaluated for resistance to powdery mildew (*Sphaerotheca macularis* f. sp. *fragariae*) using controlled greenhouse inoculations and field trials with differing natural infection levels. Genetic differences contributed a large fraction of the phenotypic variance among individuals for both disease incidence ($H^2 = 0.44$–0.71) and disease severity ($H^2 = 0.70$–0.94) in all cases, but the variance attributable to breeding value for these traits varied substantially with infection level ($h^2 = 0.12$–0.90). Likewise, genotypic and breeding value correlations for a single trait scored in different infection environments suggest that different genes may confer resistance with different levels of disease pressure. Greenhouse evaluations corresponded well with rankings obtained under high levels of field infection. However, evaluation of genetic potential only under conditions of extreme infection may ignore valuable components of partial resistance, and should not be used in isolation.

Powdery mildew of strawberry (*Fragaria* × *ananassa* Duch.) is caused by an obligate parasite, *Sphaerotheca macularis* (Wallr. ex Fr.) Jacz. f. sp. *fragariae* Peries, which can infect foliage, flowers, and fruit. The disease reduces fruit yields by lowering the productivity of infected plants and rendering infected berries unmarketable (25). Yield losses of up to 60% have been reported in the U.S. (9). In California production fields, the disease is controlled using foliar applications of sulfur, the demethylation-inhibiting fungicide myclobutanil (Rally, Rohm and Haas, Philadelphia, PA), or both. Documentation of genetic resistance development in powdery mildew populations against demethylation-inhibiting fungicides (1,3,15,18) argues for an integrated disease control approach including the employment of host resistance or tolerance.

An understanding of the inheritance of genetically controlled traits enables the plant breeder to design optimal strategies for incorporating favorable characters, such as disease resistance, into new varieties. The inheritance of powdery mildew resistance in *Fragaria* sp. has been studied in several breeding populations

(5,8,10,12,13,23) and somewhat different conclusions have been reached in each case. Despite some variation in these research outcomes, the consensus is that both additive and nonadditive variance components are important in the inheritance of mildew resistance in *F.* × *ananassa*. Furthermore, none of the reports provide convincing evidence of simple inheritance of resistance mechanism, suggesting that breeding for partial resistance may be necessary.

Some of the variation in results from genetic screening trials may trace to differing environments. The precision of field evaluations of genetic resistance is adversely affected by heterogeneous levels of natural inoculum, and selection efficiency may be improved by developing more controlled screening methods. However, the value of these alternative screening methods depends on their capability to discern genetic differences for resistance that are relevant to production environments, and the results obtained to date for strawberry powdery mildew have been contradictory. For example, McNicol and Gooding (13) found no correlation between the resistance reactions of immature (3.5-mo-old) seedlings grown in a glasshouse and the same genotypes evaluated as mature field-grown plants. Conversely, Gooding, McNicol, and MacIntyre (6) later reported a high phenotypic correlation ($r = 0.92$) between mildew resistance scores given to four strawberry genotypes grown under polyethylene tunnels and in the open field.

The first objective of our study was to estimate genetic parameters useful in describing the mode of inheritance for host resistance to strawberry powdery mildew, using a random sample of short-day and day-neutral parents from the University of California strawberry germ plasm pool. The distribution and magnitude of genetic variation is perhaps the primary determinant in development of efficient breeding and selection strategies. A second objective was to investigate the genetic relationship between resistance in mature greenhouse-grown seedlings and mature field-grown progenies from the same parents, to determine whether greenhouse screening could be used to develop elite resistant lines rapidly or to identify superior crosses or parents based on short-term progeny tests.

MATERIALS AND METHODS

Three cultivars and four advanced selections were used as parents in a seven-parent half-diallel crossing system without selfs, with four cells missing. The parents used here were a random sample of the 26 advanced-generation genotypes used in creating seedlings for selection in 1992, and had not been selected consciously for powdery mildew reaction characteristics. The 17 unique full-sib families were scored for powdery mildew infection traits at three locations in California: a Davis greenhouse, a Watsonville field site, and a Santa Maria field site. Cultural descriptions are given below for each trial.

Davis greenhouse test. Seedlings were germinated and grown for 5 wk in a Davis greenhouse, transplanted to 14-cm-diameter plastic pots and maintained in the pots for the duration of the greenhouse trial. Each family was represented by 16–20 genotypes, which were distributed among four replicates of 4–5 seedlings each. Families were randomized on each of four replicate greenhouse benches, all within one large greenhouse. Potted seedlings were watered as needed and fertilized weekly with 250 ml of a 2.4 g/L solution of soluble 20-20-20. Seedlings were inoculated 7 July 1992 as mature (11-mo-old) greenhouse-grown plants with dry *S. m. fragariae* conidia of a mass isolate collected in Watsonville, CA. Inoculations were made using a vacuum-operated settling tower to achieve uniform deposition of conidia over plant surfaces (16). The settling tower holds up to nine 14-cm potted plants per inoculation. Seedlings were systematically inoculated, one from each of nine families followed by one from each of the remaining eight families within one replicate at a time. Five uniformly infected field-collected strawberry leaflets were used for each inoculation and conidia densities averaged approximately 500 per cm^2. The seedling plants were evaluated for powdery mildew resistance 15 September 1992.

Watsonville field test. An additional 16–20 unique genotypes from the 17 crosses evaluated in the greenhouse were used for evaluation of field resistance. These genotypes were germinated and grown as for the greenhouse test, except that the seedlings were transplanted to the field on 14 September 1991 at the University of California Strawberry Research Facility, Watsonville, CA. Each cross was represented by a single plot of 8–10 seedlings in each of two replications. The plants were powdery mildew free at the time of transplanting. No fungicide treatments were applied to the seedling plants for the entire 1991–92 fruit production season. The seedlings were evaluated for powdery mildew resistance 18 October 1992.

Santa Maria field test. In November 1992 and after initial powdery mildew evaluations were complete, a subset of the seedling plants from the Davis greenhouse trial were relocated to a lathhouse and maintained over winter. On 25 February 1993 these seedlings were pruned and treated with wettable sulfur to reduce residual *S. m. fragariae* inoculum. On 1 March 1993 plants were removed from their pots, and all soil was shaken from their roots. The dormant plants were held in cold storage (1.1 C) for 1 wk and then transplanted to the field 8 March 1993 at the University of California Cooperative Extension Strawberry Research Facility in Santa Maria, CA. After establishment, 180 seedlings were available for evaluation and each family was represented by at least 7 seedlings. The seedlings were evaluated for powdery mildew resistance 12 June 1993.

Seedlings grown at all three test sites were evaluated for powdery mildew resistance traits using the following procedures: 10 medium-aged leaflets were chosen randomly from each seedling, and the abaxial surfaces were rated for the incidence (presence/absence) and severity (percentage of leaflet area infected) of disease. For the purposes of this study, disease incidence (DI) and disease severity (DS) are defined as the mean percentage of infected leaflets per seedling plant and the mean percentage of infection of the abaxial leaflet surface, respectively. The distributions of all original variables, and the cross means for all variables, were significantly skewed and leptokurtic. Because these distributional features will violate several statistical assumptions of our model, all variables were treated with a LOGIT transformation prior to performing analyses of variance (24).

Analyses of variance and covariance were conducted to test the significance of General Combining Ability (GCA) and Specific Combining Ability (SCA) using the least-squares procedure DIALL of Schaffer and Usanis (17). Replications were treated as fixed effects and interaction sums of squares and degrees of freedom between genetic sources and replications were pooled with the within-family component; this procedure was adopted to eliminate the scale differences among replications and any within-replication inoculum heterogeneity as sources of bias in genetic parameter estimates. GCA and SCA effects were tested using expectations of mean squares (Table 1). Model components of variance for GCA, SCA, and within-family sources were estimated using the restricted maximum likelihood procedure of

TABLE 1. Form and expected mean squares for diallel analysis of powdery mildew disease ratings from 17 strawberry progenies evaluated in three trials

Source	Degrees of freedom: Davis	Watsonville	Santa Maria	Expected mean squares[a]
Reps	3	1	...	$\sigma^2_w + k_3 \sigma^2_r$
GCA[b]	6	6	6	$\sigma^2_w + k_1 \sigma^2_s + k_2 \sigma^2_g$
SCA[c]	10	10	10	$\sigma^2_w + k_1 \sigma^2_s$
Error	306	303	163	σ^2_w

[a] Coefficients for Davis, Watsonville, and Santa Maria trials, respectively, are $k_1 = 19.2$, 18.7, and 14.4; $k_2 = 76.1$, 75.8, and 41.4; $k_3 = 81.6$, 160.5, and 0. σ^2_r, σ^2_g, σ^2_s, and σ^2_w are variance due to replications, general combining ability, specific combining ability, and within cross genetic plus error.

[b] General Combining Ability.

[c] Specific Combining Ability.

Huber (11). These model variance components were translated to casual genetic components and heritabilities were estimated according to Hallauer and Miranda (7), as follows:

$$h^2 = \frac{4\,(\sigma^2_{gca})}{2\,(\sigma^2_{gca}) + \sigma^2_{sca} + \sigma^2_{w}} \quad (1)$$

$$H^2 = \frac{4\,(\sigma^2_{gca} + \sigma^2_{sca})}{2\,(\sigma^2_{gca}) + \sigma^2_{sca} + \sigma^2_{w}} \quad (2)$$

In equations 1 and 2, σ^2_{gca}, σ^2_{sca}, and σ^2_{w} are variances due to general and specific combining ability and an error variance that includes within-family genetic and environmental sources (7). The assumptions required for valid translation of model components to causal genetic components have been discussed previously, both for strawberries in general (4), and for California strawberry populations specifically (20–22). These heritability estimates should be reasonably accurate in predicting short-term selection response for the California reference population.

Genotypic correlations between field traits evaluated at Watsonville and greenhouse traits were calculated as Burdon (2):

$$r_{g(xy)} = \frac{r_{c(xy)}}{\sqrt{h^2_{c(x)} \cdot h^2_{c(y)}}} \quad (3)$$

whereas, genotypic correlations between field traits evaluated at Santa Maria and greenhouse traits were calculated as Searle (19):

$$r_c = \frac{\sigma_{gca(xy)} + \sigma_{sca(xy)}}{\sqrt{[\sigma^2_{gca(x)} + \sigma^2_{sca(x)}] \cdot [\sigma^2_{gca(y)} + \sigma^2_{sca(y)}]}} \quad (4)$$

In equation 3, $r_{c(xy)}$ is the phenotypic correlation of cross means after correction for the main effects of test site, $h^2_{c(x)}$ and $h^2_{c(y)}$ are cross-mean heritabilities for field and greenhouse traits respectively, calculated as described below. Because the genotypes evaluated at Santa Maria were a subset of those evaluated in the greenhouse, genotypic correlations between traits for these samples may be calculated using direct measurements on identical genotypes. In equation 4, $\sigma_{gca(xy)}$ and $\sigma_{sca(xy)}$ are the components of covariance for traits x and y due to GCA and SCA; this value was calculated from linear functions of expected and experimental cross products obtained using analyses of cross products (17); $\sigma^2_{gca(x)}$, $\sigma^2_{sca(x)}$, $\sigma^2_{gca(y)}$, and $\sigma^2_{sca(y)}$ are model genetic variance components for traits x and y, calculated as described above.

Cross-mean heritabilities used in equation 3 were calculated as Namkoong (14):

$$h^2_c = \frac{2(\sigma^2_{gca}) + \sigma^2_{sca}}{2(\sigma^2_{gca}) + \sigma^2_{sca} + \sigma^2_w/n} \quad (5)$$

In equation 5, n is the number of seedlings per cross.

TABLE 2. Means and standard deviations (in parentheses) for disease incidence (DI) and disease severity (DS) for powdery mildew in 17 field- and greenhouse-grown strawberry progenies

Population	N	Rating	
		DI	DS
Field (Watsonville)	321	37.5 (21.5)	14.0 (16.5)
Field (Santa Maria)	180	100.0[a]	44.7 (23.3)
Greenhouse (Davis)	326	54.8 (21.3)	32.1 (23.8)

[a]All genotypes at the Santa Maria field test site were infected to some extent.

Breeding values were calculated for each of the seven parents by using the restricted maximum likelihood method of Huber (11), and product-moment correlations among breeding values (r_{bv}) were calculated between greenhouse and field DI and DS ratings using these estimates of breeding value.

RESULTS AND DISCUSSION

Overall infection level at the three test sites was highest at the Santa Maria field test (DI = 100, DS = 44.7), lowest at the Watsonville field test (DI = 37.5, DS = 14.0), and intermediate at the Davis greenhouse test (DI = 54.8, DS = 32.1) (Table 2). Infection at the Santa Maria location was so severe that all leaves scored had some level of infection, and variation for disease incidence is not evaluated further for this trial.

The significance of GCA and SCA variances, and the magnitude of h^2 and H^2, differed among the three trials and appeared dependent on the infection level of the population evaluated (Table 3). GCA variances for both DI and DS in greenhouse-grown progenies, and GCA variance for DS in the Santa Maria field-grown progenies were highly significant, whereas SCA was relatively unimportant. Conversely, SCA variances were highly significant for both DI and DS in Watsonville field-grown progenies and GCA variance was not (Table 3). Heritability estimates reflect a similar pattern, with relatively high h^2 and H^2 in trials associated with high infection levels (Table 3). Because heritabilities differed most between the two trials that shared a common inoculum source (Watsonville and Davis), it is unlikely that this pattern reflects pathogen heterogeneity. Importantly, although estimates of h^2 differ by as much as a factor of seven for a single trait scored in differing environments (h^2 = 0.12–0.90, Table 3), estimates of H^2 increased by at most 61% (H^2 = 0.44–0.71, Table 3). Increases in H^2 with higher infection levels may result in part from more uniform distribution of inoculum, and smaller associated errors of resistance score classification. However, the observation that the distribution of genetic variance components changes substantially with infection level suggests that different

TABLE 3. Results from diallel analysis of variance of disease incidence (DI) and disease severity (DS) for powdery mildew in 17 field- and greenhouse-grown strawberry progenies, together with genetic parameter estimates

Source	Watsonville field ratings (mean square)		Santa Maria field ratings (mean square)		Greenhouse ratings (mean square)	
	DI	DS	DI	DS	DI	DS
Reps	0.05	1.72	...	...	10.92**[a]	4.82*[b]
GCA[c]	6.18	9.81	...	14.20**	20.05**	37.98**
SCA[d]	2.79**	5.40**	...	1.36	0.83	1.37
Error	1.10	1.27	...	1.16	0.84	1.05
h^2	0.15	0.12	...	0.66	0.71	0.90
H^2	0.44	0.70	...	0.72	0.71	0.94
h^2_c	0.82	0.83	...	...	0.92	0.94

[a]**, Statistical significance at the 0.01 probability level.
[b]*, Statistical significance at the 0.05 probability level.
[c]General Combining Ability.
[d]Specific Combining Ability.

TABLE 4. Breeding value correlations (r_{bv}) and genotypic correlations (r_g) for disease incidence (DI) and disease severity (DS) for powdery mildew in 17 field- and greenhouse-grown strawberry progenies

Field disease ratings	Greenhouse ratings			
	DI		DS	
	r_{bv}	r_g	r_{bv}	r_g
DI low infection	0.55	0.57*[a]	0.63	0.41
DS low infection	0.55	0.60*[a]	0.52	0.43
DS high infection	0.98**[b]	0.97**[b]	0.93**[b]	0.97**[b]

[a]*, Statistical significance at the 0.05 probability level.
[b]**, Statistical significance at the 0.1 probability level.

TABLE 5. Breeding values of seven parent varieties used in the partial half-diallel crossing scheme, obtained from disease incidence (DI) and severity (DS) ratings of 17 greenhouse-grown and field-grown strawberry progenies

	Watsonville field ratings		Santa Maria field ratings	Greenhouse ratings	
Parent	DI	DS	DS	DI	DS
Selva	−0.08	0.13	0.17	0.21	0.19
Parker	−0.30	−0.15	−0.69	−0.75	−0.99
Seascape	0.05	−0.08	−0.26	−0.36	−0.54
CA 85.22-1	0.09	0.01	0.96	0.74	1.07
CA 87.109-3	0.09	0.24	−0.07	0.19	0.25
CA 87.153-2	0.18	−0.07	−0.16	−0.12	0.05
CA 88.70-613	−0.04	−0.09	0.05	0.09	−0.02

genes confer relative resistance when the plant is stressed with differing levels of disease pressure.

Genotypic correlations (r_g) and correlations among estimated breeding values (r_{bv}) for disease ratings between greenhouse and field tests are summarized in Table 4. In general, these results demonstrate that genetic differences in resistance were expressed similarly in the greenhouse-grown and field-grown progenies, but also suggest that the degree of correspondence depends on the level of infection. Moderate and occasionally significant ($P < 0.05$) correlations (r_g, r_{bv} = 0.41–0.63, Table 4) were obtained between greenhouse and Watsonville field disease ratings, in which infection of field-grown plants was low. However, highly significant ($P = 0.01$) correlations (r_g, r_{bv} = 0.93–0.98, Table 4) were found between greenhouse and Santa Maria disease ratings, in which infection levels were uniformly high. Little difference was observed between r_g and r_{bv} for any of the trait pairs, suggesting that genes with both additive and nonadditive effects for resistance are affected by infection level.

Breeding values were calculated for each of the seven parent genotypes used in the study and their ranks compared across environments (Table 5). As expected from observations on r_{bv}, the parental rankings are not entirely consistent across different trials. In general, selection of parents based on a greenhouse evaluation would rarely result in the discard of a superior parent (e.g., cvs. Parker or Seascape), and is expected to be reliable in eliminating the most susceptible types (e.g., CA 85.22-1).

Because resistance mechanisms expressed under severe infection stress are conditioned largely by the additive effects of their genes, good correspondence is expected between parental and clonal performance, perhaps obviating the need for progeny tests in choosing superior parents. The weak correspondence between genetic resistance mechanisms with differing severities of infection is reason for some caution, as it suggests that different sets of genes may confer resistance under different inoculum levels. Use of resistance expressed only under conditions of severe infection may ignore valuable components of partial resistance, and this should be the subject of further investigation.

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