Performance Responses of Strawberry Seedlings to the Sublethal Effects of Nonfumigated Soils

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Abstract. Nine selected strawberry genotypes (*Fragaria* × *ananassa* Duch.) from the University of California, Davis, strawberry improvement program were intercrossed and their seedling offspring evaluated for five production traits. Plants were evaluated in annual hill culture, with and without preplant soil fumigation using a mixture of 67 methyl bromide : 33 chloropicrin (wt/wt, 392 kg/ha). Plant mortality was <1% for seedlings grown in either soil environment, indicating that the main effects of fumigation treatment in this experiment were due to the consequences of sublethal soil organisms. Plants grown in nonfumigated soils measured from 74% to 77% of the diameter of those grown in fumigated soils and yielded 59% as much fruit. Significant cross × fumigation interactions were not detected for fruit yield, fruit size, and weighted fruit appearance. Moreover, genetic correlations for these three traits calculated by comparing seedling performance in fumigated and nonfumigated soil environments were at or near unity, suggesting that the same genes condition genetic variability for these traits in both soil environments. Together, these findings demonstrate that strawberry fruit yield and vigor are increased substantially by fumigation, even in the absence of an identifiable major pathogen problem. Further, there may be little promise for developing cultivars with genetic adaptation specific to the sublethal effects of nonfumigated soils, as selection in either soil fumigation environment is likely to affect the same sets of genes.

Many studies conducted over the last four decades have demonstrated increases in strawberry productivity following preplant soil fumigation (Himelrick and Dozier, 1991; Wilhelm and Paulus, 1980; Wilhelm et al., 1974). Soil fumigation mitigates production problems associated with weeds, nematodes, and soilborne fungal pathogens (Wilhelm and Paulus, 1980). The observed response in strawberry growth and productivity to soil fumigation can result both from the control of lethal pathogens and from control of a highly variable complex of sublethal, competitive soil organisms. Control of lethal pathogens results in reduced plant mortality, whereas control of the sublethal pathogen complex is expressed as increased plant vigor (Wilhelm and Paulus, 1980). Both of these sources of fumigation response have been observed in strawberry (Wilhelm et al., 1961; Yuen et al., 1991) and have been cited to explain the widespread use of fumigation in high-yielding strawberry production regions such as California (Voth and Bringham, 1990).

Development of cultivars with specific adaptation to nonfumigated soils has been suggested as a potential alternative to preplant soil fumigation (Watson et al., 1992). Such cultivars must possess adaptation to environments that contain both lethal and sublethal soil organisms, so information about the nature and availability of genetic variation for response to both source types is needed. However, most investigations conducted to assess genetic alternatives to soil fumigation have focused solely on finding resistance to specific lethal pathogens (Galletta et al., 1989). Recently, the potential for developing cultivars genetically adapted to the nonspecific vigor reduction of nonfumigated soils was evaluated using twelve strawberry genotypes grown in fumigated and nonfumigated soils at two California locations (Larson and Shaw, 1995). In these trials, plant mortality was less than 3% and did not differ across fumigation treatments, so the main effects

of fumigation treatment were attributed to the consequences of sublethal soil organisms. Plants raised in nonfumigated soils had uniformly lower yields and less early vegetative vigor than their counterparts grown in fumigated soils. Most importantly, genotype × fumigation interactions were uniformly nonsignificant for all traits evaluated. Thus, the relative performance of genotypes tested with differing fumigation treatments did not change: plants grown in either fumigation environment expressed variance largely conditioned by the same sets of genes.

Inferences about opportunities for obtaining strawberry cultivars adapted to the sublethal effects of soil organisms based on genotypic analyses are necessarily limited. First, conclusions frequently are drawn from testing a small number of distinct genotypes, and the estimates of genetic variation obtained from these small populations are subject to substantial genetic sampling error. Second, the genotypes chosen for study usually represent a highly selected subset, and the genetic variance among individuals within such subsets may not accurately reflect that in the base breeding population. A third concern is that selection pressure on the genotypes chosen for study may have been applied strictly through testing in fumigated soil environments. For these reasons, the selected genotypes included for testing by Larson and Shaw (1995) may not accurately reflect the adaptational variation available for nonfumigated soil conditions within the breeding population. The present study was conducted to extend the conclusions derived in the genotypic assessment of adaptational variation, specifically that of Larson and Shaw (1995), to a broader germplasm sample. As in the genotypic trial, the priority was to appraise the degree of correspondence in performance across fumigated and nonfumigated soil environments.

Materials and Methods

Seedlings from each of 19 biparental crosses among nine parental genotypes were planted at the Univ. of California Watsonville Strawberry Research Facility on 22 Sept. 1992 ac-

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cording to practices established previously for experimental seedling trials (Shaw et al., 1989). The nine parental genotypes were representative of cultivars and advanced selections within the Univ. of California breeding program. Crosses among elite selections were performed to maximize segregational genetic variation, thereby simulating the range of advanced germplasm available within the Univ. of California breeding program for obtaining further genetic advance.

A randomized complete block design with replications nested in fumigation treatments was used to test the seedling offspring from each cross. A total of 40 seedlings from each of the 19 crosses were assigned at random to either of two fumigation treatments, so that 20 seedlings per cross were assigned to each soil treatment. Fumigation treatments were 1) standard soil fumigation with 67 methyl bromide : 33 chloropicrin (wt/wt) at 392 kg/ha; or 2) no fumigation. Both fumigation treatments were applied to soils that had been cropped in strawberry in alternate years for several production cycles (Larson and Shaw, 1995), but were free of any identifiable lethal pathogen problem. The 20 seedlings per cross assigned to each treatment were further divided into two equal subsets and assigned to one of two replications, where they were established as a single experimental plot of 10 seedlings. These plots of 10 seedlings were subsequently randomized within each of the four fumigation/replication combinations.

Fruit harvests were initiated on 8 Apr. and continued for 13 consecutive weeks until 8 July. These harvests captured an estimated 85%–90% of the yield expected for the season in total. Fruit yield in grams, fruit number, and a fruit appearance score (Shaw et al., 1989) were recorded weekly for each plant. Fruit size was calculated by dividing the total yield by the total number of fruit. A weighted fruit appearance score was calculated by weighting the weekly fruit appearance scores by their corresponding weekly yields and taking the average over the course of the season. Early vegetative growth was evaluated using cross-sectional plant diameters (Shaw, 1993) obtained on 30 Nov. and 19 Feb. Large differences in scale were observed between fumigation treatments for fruit yield, and an appropriate power transformation was conducted before statistical analysis of this trait (Fernandez, 1992).

ANOVAs were conducted for all traits with fumigation treatments and replications as fixed effects and crosses as random effects, using SAS procedure GLM (SAS Inst., Cary, N.C.) Significance tests were performed using Type III expected mean squares (Table 1). Cross (σ^2_c), fumigation $\times$ cross interaction (σ^2_{fc}), and within-cross (σ^2) model variance components were estimated by equating the expected mean squares in Table 1 with their corresponding experimental mean squares, and solving the resulting linear equations. Using the complete ANOVA model described in Table 1, individual-plant heritabilities for the seedlings were calculated as:

$$h^2 = 2(\sigma^2_c)/(\sigma^2_c + \sigma^2_{fc} + \sigma^2) \quad [1]$$

for each of the five production traits. This equation returns a heritability estimate greater than the narrow-sense heritability and less than the broad-sense heritability, depending on the distribution of additive and nonadditive genetic effects (Shaw, 1993).

Genetic correlations for individual traits evaluated in different fumigation environments were calculated for each trait as (Burdon, 1977):

$$r_g = r_{p(c)} / \sqrt{[h^2_{c(fum)}] \times [h^2_{c(non)}]} \quad [2]$$

where $r_{p(c)}$ is the correlation of cross means between the two fumigation environments, and $h^2_{c(fum)}$ and $h^2_{c(non)}$ are the cross-mean heritability estimates in fumigated and nonfumigated soils, respectively. Cross-mean heritability was calculated as

$$h^2_c = \sigma^2_c / (\sigma^2_c + \sigma^2/n) \quad [3]$$

The variance components used for calculation of cross-mean heritabilities are analogous to those in Table 1, but were obtained with fumigated and nonfumigated treatments analyzed separately; n is the harmonic mean of the number of seedlings for each cross/fumigation combination and was 19.9 and 19.8 for fumigated and nonfumigated analyses respectively. The genetic correlation calculated using (2) is of particular importance: by treating a single trait evaluated in two environments as two separate but genetically correlated traits, testing options can be evaluated using indirect selection theory (Falconer, 1981). This procedure can then be used to predict the similarity of response expected when selection is performed on a single trait in one fumigation environment and performance is tested in the other.

Table 1. Expected mean squares for analysis of variance of five growth and productivity traits in the Watsonville strawberry fumigation trial.

Source	df	Expected mean squares
Fumigation (F)	1	$\sigma^2 + 188.7 \sigma^2_{f/f} + 377.0 \sigma^2_f$
Replication (R/F)	2	$\sigma^2 + 188.7 \sigma^2_{r/f}$
Cross (C)	18	$\sigma^2 + 19.9 \sigma^2_{fc} + 39.7 \sigma^2_c$
F $\times$ C	18	$\sigma^2 + 19.9 \sigma^2_{fc}$
Within-cross	715	σ^2

Table 2. Means and standard deviations (in parentheses) for five growth and productivity traits for strawberry seedlings grown with two pre-plant soil fumigation treatments at Watsonville, Calif.

	Nov. plant diam (cm)	Feb. plant diam (cm)	Yield (g/plant)	Fruit size (g/fruit)	WFA ^y
Fumigation treatment ^z					
Nonfumigated	16.0 (2.6)	17.6 (2.8)	935 (370)	19.1 (4.2)	3.00 (0.41)
Fumigated	21.5 (2.5)	22.8 (2.8)	1590 (423)	19.1 (3.5)	2.93 (0.39)

^zNonfumigated and fumigated indicate no soil fumigation and preplant soil fumigation with 392 kg/ha of 67 methyl bromide : 33 chloropicrin (wt/wt), respectively; N = 379 for fumigated treatment means, and N = 376 for nonfumigated treatment means.

^yWFA = weighted fruit appearance.

Results and Discussion

Plant mortality was <1% overall and did not differ substantially between fumigated and nonfumigated soils. As major pathogens are typically lethal in their expression, this low mortality rate indicates that the main effects of fumigation treatment were due to the consequences of sublethal soil organisms.

Plants grown in fumigated soils had larger plant diameters and greater yields than those grown in nonfumigated soils (Table 2). In November and February the average diameters of seedlings planted in nonfumigated soil were 74% and 77%, respectively, of those grown with fumigation. The fumigation response for total yield was larger than for all other traits, with seedlings grown in nonfumigated soils yielding only 59% of the fruit produced by those grown in fumigated soils. Moreover, the relative productivity of seedlings in fumigated and nonfumigated soils did not vary substantially over the 13 weeks of harvest (Fig. 1). The similarity of these fruiting patterns suggests that fumigation effects did not alter the timing of plant development and productivity. Instead, plant vigor was affected uniformly at all stages of growth and development. No differences due to fumigation treatment were noted for fruit size or weighted fruit appearance (Table 2). The

Fig. 1. Mean weekly fruit yield for strawberry seedlings grown in fumigated and nonfumigated soil.

absence of a significant fumigation effect for fruit size differs from the result obtained in a previous trial (Larson and Shaw, 1995), where nonfumigated fruit sizes at the Watsonville location were 88% of those in fumigated soils.

Differences between fumigation treatments for plant diameter and yield were highly significant ($P < 0.01$, Table 3). Highly significant among-cross variances for yield, fruit size, and weighted fruit appearance were also detected. The among-cross variances for the November and February plant diameters were significant ($P < 0.05$) and nonsignificant, respectively. Heritabilities for all traits except fruit size ($h^2 = 0.33$) were low ($h^2 < 0.15$) (Table 3). These results demonstrate the presence of genetic variability for all traits except February plant diameter, although not in excessive quantity; this might be the consequence of using a highly selected set of parents, although these parents are certainly representative of genotypes used in the California program. Importantly, the relatively low heritability was attributable primarily to a high ratio of within-cross variance (σ^2) to among-cross variance (σ^2_c); no significant fumigation $\times$ cross interaction variance (σ^2_{fc}) was present for any trait except February plant diameter (Table 3).

The virtual absence of fumigation $\times$ cross interaction variance for the traits evaluated in this study indicates that genetic differences among crosses were expressed consistently across fumigation environments. Quantitative verification of this conclusion is evident through examination of individual trait expressions in fumigated and nonfumigated soil environments as separate but genetically correlated. Genetic correlations (r_g) were at or near unity for yield, fruit size, and weighted fruit appearance (Table 4), indicating that the same sets of genes condition genetic variability for these traits in both fumigated and nonfumigated soils. The significant ($P < 0.05$) fumigation $\times$ cross interaction variance and low r_g (0.31) for February plant diameter appears to be of limited importance, considering that no significant among-cross (genetic) variation was detected for this trait and no consequences for yield were evident.

The relative consistency of gene expression across fumigation environments is in agreement with the results obtained for genotypic

trials conducted simultaneously (Larson and Shaw, 1995). Together, the results for the genotypic and seedling studies support an interpretation whereby the genes that confer superior performance in fumigated soils will confer similar levels of relative response in nonfumigated soils in the absence of a lethal pathogen problem. Furthermore, because testing for improved performance in nonfumigated soils could increase error due to added heterogeneity of the soil environment, procedures that depend on screening new or advanced selections in such soils might suffer from reduced efficiency (Larson and Shaw, 1995). As a consequence, there appears to be little or no advantage to initiating a cultivar improvement program directed at breeding in nonfumigated soils for specific genetic adaptation to the sublethal effects of soil organisms, at least within this germplasm base. The possibility that other germplasm sources may express mechanisms for adaptation to the sublethal consequences of soil organisms should be explored. However, utilization of germplasm outside the California program may have substantial costs (Larson and Shaw, 1995; Rariden and Shaw, 1994).

Although mortality due to lethal pathogens is rare in California strawberry production fields at present, this would not be anticipated with repeated cropping cycles in the absence of effective soil fumigants. When a lethal pathogen is of primary importance, its presence or absence constitutes different production environments. In such cases, the occurrence of the pathogen in combination with discrete resistance classes (or even large metric differences) can lead to large rank shifts in genotypic performance (Baker, 1988). Thus, although lethal and sublethal pathogens both may contribute to reduced strawberry plantation performance in nonfumigated soils, their potential genetic solutions differ dramatically, and should be distinguished when formulating breeding strategies.

Considering these multiple objectives, a prudent course of action at this time might be to test and select for most production traits in fumigated soils first and then test for resistance to specific pathogens later. This two-stage culling strategy would avoid elimination of outstanding individuals that may lack specific resistance required only in certain production environments. The order of selection is important to avoid loss of useful genotypes due to excessive disease pressure. Regardless of the soil environment for intended production, there seems little reason to transfer strawberry selection procedures to nonfumigated soils.

Table 3. Results of analysis of variance for five growth and productivity traits for strawberry seedlings grown with two preplant soil fumigation treatments at Watsonville, Calif.

Source	Mean squares				
	Nov. plant diam (cm)	Feb. plant diam (cm)	Yield (g/plant)	Fruit size (g)	WFA ^z
Fumigation (F)	5707.28**	5262.41**	1216.48**	0.00	88.55
Replication (R/F)	12.44	1.20	11.73**	91.49**	19.85
Cross (C)	25.20*	19.83	9.06**	132.70**	67.58**
F $\times$ C	10.51	14.21*	2.42	5.81	18.73
Within-cross	6.57	7.78	2.39	15.04	15.84
σ^2	0.37	0.14	0.17	3.19	1.23
σ^2_c	0.20	0.32	0.00	-0.46	0.15
h^2_{fc}	0.10	0.03	0.13	0.36	0.14

^zWFA = weighted fruit appearance.

*,** Significant at $P = 0.05$ or 0.01 , respectively; mean squares and variance components for yield have been divided by 1000 and components for weighted appearance have been multiplied by 100 for ease of presentation.

Table 4. Separate analysis of variance results for five growth and productivity traits for strawberry seedlings grown with or without preplant soil fumigation at Watsonville, Calif.

Fumigation treatment	Source	Mean squares				
		Nov. plant diam (cm)	Feb. plant diam (cm)	Yield (g/plant)	Fruit size (g)	WFA ^z
Nonfumigation	Replication	15.84	2.19	1967.07	182.61**	2.13
	Cross	16.98**	18.94**	6195.45**	75.66**	24.96
	Within-cross	6.76	7.86	2406.28	17.52	16.58
	h ² _c	0.60	0.59	0.61	0.77	0.34
Fumigation	Replication	9.04	0.01	21501.34**	0.37	37.57
	Cross	18.76**	15.09*	5288.63**	62.89**	61.59**
	Within-cross	6.39	7.71	2378.96	12.58	15.10
	h ² _c	0.66	0.49	0.55	0.80	0.75
NF vs. F	r _{pc}	0.41	0.16	0.58	0.92	0.60
	r _g	0.65	0.31	1.00	1.17	1.20

^zWFA = weighted fruit appearance.

*** Significant at $P = 0.05$ or 0.01 , respectively; mean squares and variance components for weighted appearance have been multiplied by 100 for ease of presentation.

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