Effects of Soil Fumigation with Methyl Bromide and Chloropicrin on Root Health and Yield of Strawberry

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ABSTRACT

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Soil fumigation with methyl bromide and chloropicrin (MBC) reduced the severity of black root rot of strawberry in experiments at four locations in California. At three of these sites, fumigation increased root density by 19–61% over the untreated controls. Improvement of root health by fumigation was correlated with increased yields of berries at all four locations. Accumulative harvests from fumigated plots were 24–29% greater than the untreated controls. At a fifth location, fumigation with MBC had no significant effect on root growth, root rot severity, or yields, and this may have been partly attributable to low disease levels. In comparison to MBC, preplant soil applications of metham-sodium and metalaxyl had no effect on root disease levels, root development, or yield. Commercial strawberry cultivars compared in experiments at two locations responded similarly to soil fumigation with MBC. Black root rot was the only disease of significance in all locations; *Cylindrocarpon destructans*, *Pythium ultimum*, and *P. irregulare* were the fungi isolated most often from diseased plants.

Soil fumigation with mixtures of methyl bromide and chloropicrin (MBC) plays a vital role in commercial strawberry (*Fragaria* × *ananassa* Duchesne) production in California. Along with high-yielding cultivars bred specifically for California environments, fumigation with MBC has been responsible for the attainment of the highest per acre yield in the nation—22 metric tons in 1988—and an annual total crop value of approximately $380 million (1).

In 1961, fumigation with MBC was reported by Wilhelm et al (19) to control Verticillium wilt of strawberry. Since then, it has been used as a preplant eradicant of weeds, nematodes, and soilborne pathogens in nearly all of the commercial strawberry plantings in California. The effectiveness of MBC in root disease control minimized the need for developing resistance to root disease in strawberry plants and hastened the development and deployment of cultivars selected primarily for fruit quality and production (18).

Methyl bromide and chloropicrin are restricted-use pesticides and are under review at the state and federal levels for possible health and environmental hazards. However, no viable alternative methods are available for managing root diseases in strawberry and for maintaining the current high production levels attained by the use of MBC. Because commercial strawberry cultivation in California during the last two decades has been almost exclusively in fumigated soils, there is little current information on the prevalence of root diseases in this state and the impact of root diseases on yields of current cultivars. This information is needed to provide a focal point in the search for alternatives. In response to these needs, we began a program of field experimentation in 1986 to compare the performance of current commercial cultivars in fumigated and nonfumigated soils and to test other fungicidal compounds (metham-sodium and metalaxyl) as alternative soil treatments. Additional objectives were to quantify the effects of fumigation and fungicide treatments on root growth and disease levels in the root system and to monitor changes in the population levels of soilborne pathogens and other root-associated fungi after soil treatments.

We report our findings on the effects of the soil treatments on root growth dynamics, root disease severity, and yield. Some of the data have been published in an abstract (20).

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MATERIALS AND METHODS

Experimental designs and soil treatments. Five experiments were conducted in the central coast region of California (Monterey, Santa Clara, and Santa Cruz counties) to test the effects of fumigation with methyl bromide and chloropicrin, as well as other soil treatments. One site was established in 1986 at the Deciduous Fruit Experiment Station in San Jose (SJ) where tomato had been planted previously and there was no recent history of strawberry cultivation or soil fumigation. Four experiments were conducted in the vicinity of Watsonville, beginning in 1987—three were located in commercial fields (CY, KS, and DSA), and one was located at the University of California Strawberry Experiment Station (WAT). Sites CY, KS, and WAT had been cropped previously with strawberry. There were fallow plantings of barley immediately before the start of experiments at KS and WAT. Strawberry was planted at site CY before the experiment. There was no previous cropping with strawberry at location DSA, where crucifers and lettuce were grown previously. Classifications of the soils, cultivars employed, planting dates, and rates of application of soil treatments are listed in Table 1.

In location SJ, metham-sodium (Soil-Prep., Wilbur-Ellis Co., Fresno, CA) and metalaxyl (Ridomil 2E, Ciba-Geigy Corp., Greensboro, NC), in addition to MBC, were tested as preplant soil treatments. The plots were arranged in a randomized block design with five replications per treatment, each occupying two adjacent beds (3 m long × 9.3 m wide) containing 15 plants per bed. MBC was injected into preformed beds under polyethylene tarp 60 days before planting. Metalaxyl and metham-sodium were applied through a low-pressure drip irrigation system (15) 7 days before planting. This procedure required that the two materials be diluted with water to a total liquid volume of 39 L per bed. This same volume of water was injected into fumigated and untreated control plots.

In the experiment at WAT, MBC and metham-sodium were tested individually on three strawberry cultivars in a split-plot design with soil treatments as the main plots and cultivars as subplots. Each soil treatment-cultivar combination was replicated five times. Plots were 4.6 m long × 0.6 m wide and contained 27 plants in double rows. Soil treatments were applied to 6 × 9 m areas before the formation of beds. MBC was injected 40 days before planting, and the entire field was covered with polyethylene tarp. Eight days after fumigation, tarps were removed from areas designated for treatment with metham-sodium. This material was applied through a sprinkler manifold consisting of six high-volume spray nozzles (Toro Co., Riverside, CA) mounted in line 1.1 m apart on PVC

tubing. The manifold was connected to the main irrigation line. When water was applied through the manifold at a pressure of 2.1 kg/cm^2 (30 psi), relatively even coverage of the treatment areas with liquid was obtained. Water alone was applied first through the sprinkler system to moisten the soil to a depth of approximately 30 cm. Metham-sodium was then injected through the manifold under pressure with additional water to carry the liquid front in the soil to a depth of 38 cm. The final concentration of metham-sodium applied in this manner was 935 L/ha. During this process, fumigated and untreated control plots were left covered with polyethylene tarp to prevent exposure to metham-sodium. Before planting, all tarps were removed and five 9-m-long beds were formed in each main plot area. The middle three beds were planted, each to one of the three cultivars tested.

Experiments at CY, KS, and DSA were designed as paired plots comparing fumigation and no fumigation. These experiments were conducted in commercial strawberry fields, and the standard commercial fumigation with MBC was applied. Sections of each field were not treated. In adjacent fumigated and untreated areas, lengths of bed were designated as treated and untreated plots, respectively. These were separated from each other by three to four planted rows. At sites CY and KS, there were four and five such pairs of beds, respectively, and each bed was 3 m long and contained 27 plants. At location DSA, sections of beds containing three adjacent rows, each 7.6 m long, in the fumigated and corresponding untreated areas were chosen as replications. Each row contained one of three different cultivars assigned at random. This arrangement enabled a comparison of a single cultivar in fumigated and nonfumigated soils and a comparison of cultivars within a soil. There were four replications for each soil treatment-cultivar combination. The planting density for each cultivar was different to allow for normal commercial cultivation practices; Chandler, Commander, and Swede were planted at 42, 38, and 50 plants per 7.6 m of bed, respectively.

Standard commercial fertilization and pest control regimes were followed at all locations. Removal of weeds by hand was required in nonfumigated plots. Plants were irrigated through T-tape drip systems, except at SJ, which had furrow irrigation.

Measurement of root growth and disease severity. Standardized root samples were obtained for root growth and root health analysis by collecting soil core samples at 2- or 3-mo intervals. Each 2.5-cm-diameter core was taken to a depth of 20 cm, 5 cm from the crown of a strawberry plant. For each replicate plot, cores were obtained from eight to 10 plants, pooled, and refrigerated up to 4 days before analysis in the laboratory.

To quantify root growth, root length density (centimeters of root per gram of soil) was measured. Root segments were extracted by wet-sieving from weighed soil core samples, and the total length of the root segments in each sample was estimated by the Newman line intersection method (10) as modified by Hancock (4).

Individual root segments were also examined under a dissecting microscope and rated as healthy, diseased (having discrete lesions or areas of discoloration), or dead (tissues collapsed or decayed). The frequencies at which each of the three disease categories occurred were used as the relative measure of root disease severity. A minimum of 100 root segments from each sample were rated.

Occasionally, entire plants were dug up and the roots were washed free of soil to examine the root systems as a whole. Plants were removed from border rows or from the margins of plots in order not to interfere with yield measurements.

Yield determinations. In experiments at SJ and WAT, ripe berries were harvested, weighed, and counted on a weekly basis. The average mass of berries was estimated either from the total harvest in each plot or from a subsample of marketable berries in each plot. Similarly, yields in experiment DSA were measured on a weekly basis; yields at CY and KS were recorded only during two 2-wk periods.

All experiments were maintained through one fruiting season. The duration varied among the experiments depending upon the site, the cultivars planted, and time of planting.

Isolation of fungi. Diseased rootlets extracted from core samples and root and crown tissues from whole plants removed from the field were cultured to isolate fungi. Root segments were washed under running tap water for 30 min, and crown tissues were surfaced-sterilized in 5% commercial bleach before culturing. Isolations were made with water agar, pectate medium (5), and cornmeal agar containing benomyl (50 mg a.i./L) and penicillin (100 mg/L). The latter was intended primarily for isolating species of *Phytophthora*. Putative pathogens were subcultured on potato-dextrose agar for purification and identification.

Statistical analysis. All data except those obtained by rating root health were analyzed by analysis of variance with mean separation through Duncan's multiple range test. Root health data were analyzed with the chi-square method. Data from replications within treatments first were tested individually to confirm homogeneity and then pooled in testing for independence.

RESULTS

Symptomatology and causal agents. An increase in plant growth was observed

Table 1. Classification of soils, cultivars employed, planting dates, and rates of application of soil treatments on strawberry fields in California

Experiment location	Soil classification	Cultivar	Planting date	Application rates of soil treatment[a] MBC (kg/ha)	Metham-sodium (L/ha)	Metalaxyl (kg a.i./ha)
San Jose (SJ)	Campbell silty loam[c]	Chandler	27 August 1986	370 (2:1)	467[b]	1.9[b]
Watsonville (CY)	Conejo loam[d]	Pajaro	27 August 1987	392 (3:2)	...	...
Watsonville (KS)	Elder sandy loam[e]	Pajaro	25 August 1987	449 (3:2)	...	...
Pajaro (DSA)	Salinas clay loam[f]	Chandler	12 November 1987	449 (2:1)	...	...
		Commander	12 November 1987			
		Swede	24 November 1987			
Watsonville (WAT)	Baywood loamy sand[g]	Chandler	3 November 1987	449 (3:2)	935[b]	...
		Oso Grande				
		Selva				

[a] Ratio in parentheses is the w/w ratio of methyl bromide to chloropicrin. All fields had untreated (control) sections.
[b] At location SJ, metham-sodium and metalaxyl were diluted in water and injected into the soil through a low-pressure drip irrigation system. At location WAT, metham-sodium was applied onto soil surface through sprinkler system after the application of water to wet soil to a depth of 30 cm.
[c] Fine-silty, mixed, thermic Aquic Xerothents.
[d] Fine-loamy, mixed, thermic Pachic Haploxerolls.
[e] Coarse-loamy, mixed, thermic Camulic Haploxerolls.
[f] Fine-loamy, mixed, thermic Pachic Haploxerolls.
[g] Sandy, mixed, thermic Entic Haploxerolls.

at some of the locations in plots that were fumigated with MBC. Differences in plant size and vigor between fumigated and untreated plots in the summer-planted (August and September) sites—CY, KS, and SJ—were noticeable within 2 mo after planting and then became less apparent by the following spring. Beginning in May and June, after 1–2 mo of fruiting, plant vigor in untreated plots continued to decline. The decline was most pronounced at location SJ and resulted in significant mortality in some of the untreated plots by the end of the experiment. In the two winter-planted sites, DSA and WAT, no differences in plant growth between treatments were detected.

Symptoms in the root system of plants in nonfumigated soils were indicative of black root rot, i.e., blackening and necrosis of cortical tissues and death of feeder rootlets. When root systems of whole plants dug from fumigated and nonfumigated areas were compared, there was no difference in the amount of structural roots. However, there was a marked lack of feeder rootlet development in plants from untreated soils.

Isolation from necrotic rootlets and crowns of plants from nonfumigated plots consistently yielded *Cylindrocarpon destructans* (Zinssmeister) Scholten, *Pythium ultimum* Trow, and *P. irregulare* Buisman. Occasionally, *Rhizoctonia solani* Kühn, binucleate *Rhizoctonia* spp., *Phoma* spp., and *Alternaria* spp. were also isolated.

Discoloration of vascular elements and of the stele, indicative of Verticillium wilt and red stele, respectively, was not seen in any experiment. *Verticillium* and *Phytophthora* were not detected in any of the root and crown isolations.

Effects of fumigation on root development and disease. Fumigation with MBC resulted in greater root growth, measured as increased root length density, in three locations—SJ, CY, and DSA (Table 2). When averaged over four measurements taken in each experiment, the increases ranged from 19 to 158%. There were no significant effects from soil fumigation on root growth at KS and WAT.

In locations DSA and WAT, in which different cultivars were tested, differences in root length density among cultivars were statistically significant, with Chandler having the highest levels in both experiments (*data not shown*). There were no significant treatment × cultivar interactions.

Root growth varied markedly over time in all locations. As typified by the trends shown at site CY (Fig. 1), root length density increased through the spring to a peak in early summer and decreased during late summer. Differences between fumigated and nonfumigated plots were most evident during the period of peak root growth. By the end of the fruiting season in mid- to late summer, these differences were not as apparent. Location SJ was the exception; significantly higher levels of root length density were maintained in the fumigated plots for more than 1 yr after treatment.

The severity of root necrosis was lower in fumigated plots than in the controls in all locations except WAT (Table 2), and a significantly larger proportion of root segments were healthy. In general, more than 50% of the root segments collected from fumigated plots were healthy when averaged over the entire season, whereas less than 40% were healthy in the controls. In location WAT, the proportions of healthy root segments in all of the three cultivars in untreated soils were relatively high, thus, fumigation had no significant effect.

In all experiments, the severity of root rot changed over time. As typified by measurements from location KS (Fig. 2), the proportions of healthy root segments in both fumigated and untreated plots were greater in earlier samplings and declined during the season with concurrent

Table 2. Root length densities and frequencies of healthy, diseased, and dead root segments measured at five locations in California comparing preplant soil treatments with methyl bromide and chloropicrin (MBC), metham-sodium, and metalaxyl

Location	Cultivar	Soil treatment[a]	Root length density (cm root per g soil)[b]	Total root segments observed (%)[b]		
				Healthy	Diseased	Dead
San Jose (SJ)	Chandler	MBC	4.75*[c]	68	16	17**[d]
		Metham-sodium	2.90	39	20	41
		Metalaxyl	1.57	35	24	41
		NT	1.84	39	22	40
Watsonville (CY)	Pajaro	MBC	3.20*	52	34	16**
		NT	2.01	26	42	31
Watsonville (KS)	Pajaro	MBC	2.87	53	30	17**
		NT	2.48	38	37	25
Pajaro (DSA)	Swede	MBC	1.56	74	20	6**
		NT	1.47	55	36	9
	Commander	MBC	1.90	78	16	6**
		NT	1.55	58	31	11
	Chandler	MBC	2.11	80	15	5**
		NT	1.66	65	22	13
	All cultivars (mean)	MBC	1.86*	77	17	6**
		NT	1.56	59	30	11
Watsonville (WAT)	Chandler	MBC	2.13	76	17	6
		Metham-sodium	1.87	66	25	10
		NT	1.82	71	20	9
	Oso Grande	MBC	1.54	66	26	9
		Metham-sodium	1.56	54	36	10
		NT	1.89	57	33	10
	Selva	MBC	1.25	74	18	9
		Metham-sodium	1.53	62	23	15
		NT	1.20	65	23	12
	All cultivars (mean)	MBC	1.64	72	20	8
		Metham-sodium	1.65	61	28	12
		NT	1.64	64	25	10

[a] NT = No treatment.

[b] Root length density was determined by measuring total length of roots, extracted from weighed soil core samples, with the line intersection method. Numbers of healthy, diseased (discolored or necrotic), and dead root segments were obtained by visual rating of individual root segments from the same samples. Values in experiments SJ and KS represent means of five replications sampled four times; in CY and DSA, four replications sampled four times; in WAT, five replications per cultivar sampled three times.

[c] * = Significant difference from untreated control at $P = 0.05$.

[d] ** = Significant difference in the frequency distribution from the untreated control at $P = 0.01$ based upon chi-square analysis of pooled data.

increases in diseased and dead segments.

Effects of fumigation on yield. In four locations (CY, KS, SJ, and DSA) out of five, fumigation with MBC resulted in significant increases in the yield of berries (Table 3). Where accumulated yields were recorded (SJ and DSA), fumigation resulted in increases in total weight of berries ranging from 22 to 28%. At locations CY and KS, where only interim harvests were measured, yields in fumigated plots were 214 and 127% greater, respectively, than in untreated plots. There were greater numbers of berries in fumigated plots in the four locations. In location SJ, the mass of individual berries was also increased by fumigation.

At location WAT, fumigation did not greatly affect total yields, individual fruit weights, or numbers of berries. Total yield of Selva in fumigated plots was 20% greater than in controls, but the difference was not statistically significant ($P = 0.05$).

Effects of other soil treatments. Preplant applications of metham-sodium to soil did not affect root growth (Table 2), disease severity (Table 2), or yields (Table 3), either when applied through a drip system in location SJ or when broadcast through a sprinkler system at WAT. Metalaxyl, applied in location SJ, also did not enhance root growth or yield or reduce root rot.

DISCUSSION

In the absence of fumigation, root necrosis, typical of the black root rot complex, became a serious problem in all experimental locations. Soil treatments with metham-sodium and metalaxyl were not effective in reducing the severity of root rot despite the fact that the treatments significantly changed population densities of fungal pathogens and other soil- and rhizoplane-inhabiting fungi (20). The lack of efficacy of metham-sodium in our experiments contrasts with findings from other studies that metham-sodium reduced inoculum levels of soilborne pathogens and thereby stimulated plant growth (2,11,14). This discrepancy perhaps is related to the involvement of different microfloras. We could not attribute the root necrosis to any one specific causal agent. Several species of fungi and nematodes are thought to be involved in the black root rot complex (8). Among the fungi, *P. ultimum* (17) and binucleate *Rhizoctonia* spp. (9) have been found to be pathogenic to strawberry. Failure of metalaxyl to reduce root rot in this study is an indication that other pathogens, in addition to *P. ultimum*, were involved. Binucleate *Rhizoctonia* spp., which are found commonly on roots of strawberry plants maintained more than 1 yr (9), were isolated very infrequently in this study. This probably was because the plants examined in our study were less than 1 yr old. The lesion nematode, *Pratylenchus penetrans* (Cobb) Filip. & Schuur.-Stek, also has been shown to cause root rot (3) and to increase the severity of root rot attributable to binucleate *Rhizoctonia* spp. (7). Because nematode populations levels were not examined in this study, the involvement of *P. penetrans* cannot be ascertained. *C. destructans* was isolated frequently

Table 3. Effects of soil fumigation with methyl bromide and chloropicrin (MBC) and preplant soil applications of metham-sodium and metalaxyl on the yield of strawberry in five field experiments in California

Site	Cultivar	Treatment[a]	Weight per plot (kg)[b]	Berries per plot (no.)[b]	Weight per berry (g)[c]
San Jose (SJ)	Chandler	MBC	12.0*[d]	938*	9.4*
		Metham-sodium	8.9	741	7.3
		Metalaxyl	8.6	654	8.1
		NT	9.3	727	7.5
Watsonville (CY)	Pajaro	MBC	20.4**	...	...
		NT	6.5	...	...
Watsonville (KS)	Pajaro	MBC	12.7**	...	...
		NT	5.6	...	...
Pajaro (DSA)	Swede	MBC	54.1*	2,386*	23.7
		NT	44.1	2,019	23.4
	Commander	MBC	65.9**	2,705**	25.4
		NT	52.8	2,276	24.5
	Chandler	MBC	21.1*	1,075*	19.1
		NT	16.7	815	19.4
Watsonville (WAT)	Chandler	MBC	30.5	...	25.5
		Metham-sodium	28.9	...	24.2
		NT	29.2	...	25.6
	Oso Grande	MBC	30.4	...	27.2
		Metham-sodium	32.7	...	26.1
		NT	31.1	...	27.4
	Selva	MBC	23.6	...	23.0
		Metham-sodium	27.3	...	24.0
		NT	19.6	...	20.3

[a] NT = No treatment.

[b] Values for experiments CY and KS are based on eight harvests conducted over two 2-week periods. Values for experiments SJ and WAT are accumulative yields from nine and 20 harvests, respectively, taken at weekly intervals. Yields expressed for experiment DSA are accumulations of 27 harvests conducted at 3- or 4-day intervals. In experiments SJ and WAT, yields of all ripe fruit regardless of size and quality were recorded, whereas only yields of marketable fruit were recorded in the other experiments. The number of plants per plot varied among the experiments.

[c] Fruit weights in experiment WAT were based on subsamples of 10 berries per replication in each harvest and on the entire yield of marketable berries in experiment DSA. In SJ, the entire yield from each harvest, regardless of fruit quality and size, was used to calculate fruit size.

[d] *, ** = Significant difference at the 95 and 99% confidence levels, respectively, from the corresponding control.

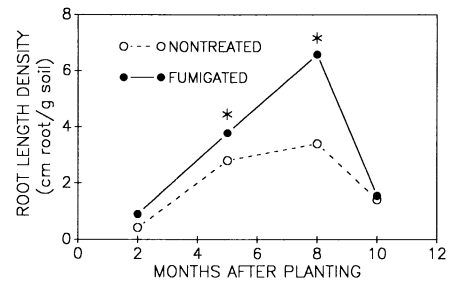

Fig. 1. Root length density of strawberry in location CY as affected by soil fumigation with methyl bromide and chloropicrin (3:2 w/w; 448 kg/ha) and by no treatment. Cultivar Pajaro was planted on 27 August 1987, 40 days after fumigation. Total lengths of roots, extracted from 2.5-cm-diameter soil core samples, were measured with the line intersection method. Values are means of four replications. * = Significant difference from the untreated control on that sampling date ($P = 0.05$).

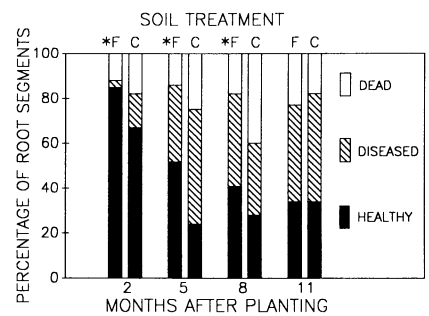

Fig. 2. Frequencies of healthy, diseased (discolored or necrotic), and dead strawberry root segments measured at location KS after soil fumigation with a 3:2 w/w mixture of methyl bromide and chloropicrin (F) at 448 kg/ha as compared with no treatment (C). Individual root segments, extracted from 2.5-cm-diameter soil core samples, were rated visually. Values represent means of five replications. * = Significant difference between the treatment and the control on that sampling date at the 99% confidence level based upon chi-square analysis. Cultivar Pajaro was planted on 25 August 1987, 35 days after fumigation.

from necrotic root and crown tissues in our experiments, and similar observations of *Cylindrocarpon* spp. were reported in other studies (6,16). However, the role of *Cylindrocarpon* spp. in the etiology of black root rot remains to be determined.

We observed a close relationship between reduction of root rot by soil fumigation and stimulation of yield. In the four locations in which yields were increased by fumigation, there were visible reductions in the extent of damage to root tissues in fumigated plots before fruiting. At a fifth experimental site (WAT), in which fumigation did not significantly affect yield, root health was not improved by fumigation. Low root disease levels in the controls may have contributed to the apparent lack of efficacy at this location. Stimulation of crop yields by fumigation, as shown in studies using shorter season annual crops, such as wheat, has been attributed to temporary changes in nitrogen availability (12). This aspect was not addressed in this study; however, because the effects of fumigation on root health in our experiments were so striking and the benefits of soil fumigation in location SJ lasted more than 1 yr (*data not presented*), we believe that control of root disease was the primary factor in the stimulation of yield.

In our experiments, yield losses of 20–25% occurred in the absence of soil fumigation. In the commercial production of strawberry, part of this loss may be recovered by savings in cost of soil treatment, which exceeds $1,000 per acre. However, if root disease is not controlled, the maintenance of ideal conditions for the growth of strawberry plants and for fruit production will be even more critical, and this will require increased inputs for weed and pest control. We have observed that with combined stress from root disease and heavy feeding by mites, there can be an exceptionally high incidence of plant mortality (G. Y. Yuen, *unpublished data*). In the absence of fumigation, it is essential that cultivars with tolerance to root diseases be employed. Cultivars tolerant to Verticillium wilt and red stele are available (13). Among the six commercial cultivars tested in our experiments, there was no evidence of tolerance to black root rot. The effect of black root rot on the yield of Pajaro, one of the most widely planted cultivars in the central coast region of California, was particularly severe. This was not unexpected, as these cultivars were bred primarily for high fruit quality and production and were not intended for culture in nonfumigated soils. There is a great need to increase the emphasis on black root rot tolerance as a selection criterion in strawberry breeding. In this regard, we found the determination of extent of root necrosis and discoloration and the measurement of root density to be better measures of overall plant health than visual assessments of aboveground growth and to provide data that was predictive of yield. Therefore, these evaluations should be useful when screening breeding lines for tolerance to black root rot and other root diseases.

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