Interactive Effects of Broccoli Residue and Temperature on *Verticillium dahliae* Microsclerotia in Soil and on Wilt in Cauliflower

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ABSTRACT

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The effects of dry, fresh, and no broccoli amendments on *Verticillium dahliae* microsclerotia in soil were evaluated at 10, 15, 20, 25, 30, and 35°C. Aliquots of 25 g of field soil naturally infested with *V. dahliae* microsclerotia (80 to 100 per gram of soil) were placed in plastic bottles to which dry (1% wt/wt), fresh (8.7% wt/wt), or no broccoli treatments were randomly assigned. One randomly chosen set of each treatment was assigned to the above temperatures and incubated for 45 days. The soil was subsequently assayed for microsclerotia using the Anderson sampler technique. The experiment was repeated nine times. At all temperatures, both dry and fresh broccoli significantly ($P < 0.05$) reduced the number of microsclerotia in soil compared with the unamended soil. At ≤30°C, fresh broccoli had a significantly ($P < 0.05$) greater remissive effect than dry broccoli. At 35°C, however, the number of *V. dahliae* microsclerotia were significantly ($P < 0.05$) reduced after 45 days in unamended soil; both fresh and dry broccoli almost completely eliminated the pathogen from soil. Temporal dynamics of the *V. dahliae* microsclerotia as affected by the treatments at the above temperatures were determined in nine experiments identical to the above by sampling and assaying soil three times at 15-day intervals. Regardless of the temperature, maximum reductions in the number of microsclerotia in treatments involving broccoli occurred within 15 days, with fresh broccoli providing significantly ($P < 0.05$) greater reductions. Further reductions in the number of microsclerotia were not significant in broccoli treatments at the subsequent sampling dates. In unamended soil, the number of microsclerotia at all temperatures except 35°C changed little throughout the experiment. The optimum temperatures for the broccoli-mediated reductions in *V. dahliae* microsclerotia in both experiments were 25 and 30°C. Four greenhouse experiments were conducted to test the effectiveness of broccoli treatments in reducing wilt incidence in cauliflower. Consistently, cauliflower plants in the fresh broccoli treatment were taller, had greater root and shoot weights, were significantly more robust, and had the least number of infected plants (number of microsclerotia was lower) than in other treatments. The number of infected plants in the dry broccoli treatment was intermediate, similar to the number of microsclerotia. For maximal reductions in soilborne *V. dahliae* microsclerotia and the subsequent lower wilt incidence in cauliflower, the broccoli residue incorporation should occur when the temperatures are at least 20°C.

Additional keywords: crop rotation, crucifer residue, epidemiology, soilborne diseases.

Verticillium dahliae Kleb. has a worldwide distribution (34), causing wilt on a broad range of crops (35). The pathogen is widely distributed in agricultural soils in California and affects such diverse crops as artichoke, cotton, pepper, pistachio, potato, strawberry, tomato, watermelon, and a number of crucifer crops. Characteristically, *V. dahliae* is a nonaggressive soil resident that seldom moves more than a few millimeters from a propagule base in undisturbed soil (16). The pathogen survives in the soil as microsclerotia for up to 10 years (14).

The key to managing Verticillium wilt is to reduce number of microsclerotia in soil to levels below which the disease does not develop on susceptible crops. This has been accomplished with a combination of chemical (43) and cultural methods (8,16). In most crops, genetic resistance is not available for Verticillium wilt. Solarization of soil has been used to reduce the number of propagules in cotton fields (16,31), but is restricted to warm, sunny climates. The imminent loss of fumigants such as methyl bromide leaves few alternatives for effective management of Verticillium wilt in crops where chemical methods are affordable.

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The effects of crop rotation on the suppression of soilborne diseases (4,5) and on maintaining crop yields (7) have been recognized and exploited for centuries. In recent decades, the use of rotations as an agronomic tool has been largely neglected by vegetable growers, because of the availability and cost-effectiveness of chemical fertilizers, pesticides, and soil fumigants. No single management practice is generally more effective in the elimination of root pathogens than crop rotation (6). Because of the wide distribution of *V. dahliae*, persistent survival of pathogen microsclerotia, lack of host specificity, and the incompatibility of graminaceous crops with current production practices in the coastal valleys of California, traditional rotations (8) are unlikely to be successful for control of Verticillium wilt.

Certain crop residues (3,8,13,20,26,28,32,33) and soil amendments (3,19,27) reduce the number of soilborne pathogen propagules and cause a concomitant decrease in the incidence of the diseases they cause (3,20,28,32,33). Interest in this area has been rekindled, because of the recent emphasis on sustainable agriculture. In particular, crucifer residues have been shown to possess disease suppressive characteristics in a number of host pathosystems (3,13,15,18,20,23,25–28,32,40). These effects have been attributed to the chemical breakdown of glucosinolates, the characteristic constituents of crucifer crops (2,9,17,22).

In general, members of the Brassicaceae contain glucosinolates that are responsible for the characteristic pungent odor in these

genera (2,9,10,22). During the decomposition of crucifer tissues, the glucosinolates break down to produce sulfides, isothiocyanates, thiocyanates, and nitriles that have either fungistatic or fungicidal properties (9,13,17) and also reduce the shelf-life of vegetables (9,11).

As early as the late 1930s, the toxicity of mustard oils and their breakdown products were demonstrated on *Colletotrichum circinans*, *Botrytis alii*, *Aspergillus niger*, *A. alliaceus*, and *Gibberella saubinetii* (42) in laboratory studies. Subsequent studies have also demonstrated the toxicity of crucifer residues or glucosinolate breakdown products on a range of plant pathogens including nematodes (13,18,23,25–28,32,41). The types and amounts of glucosinolates vary with the crucifer species (10,17,23) and determine the level of plant pathogen growth reduction. Allyl isothiocyanate production in crucifer crops, which has been shown to be effective against many pathogens (13,23), is higher with higher levels of sulfur nutrition in soil (12). Soil amendments with crucifer crop residue when combined with solarization produce a greater variety of toxic compounds (13,32) than with crucifer residue alone, and this combination may be effective on a wide variety of plant pathogens and improve the effectiveness of solarization (20). In the coastal valleys of California, however, temperatures are seldom high enough to achieve effective solarization of soil.

The disease suppressive effects of crucifer residues are also related to the degree of dryness of the crucifer residue at the time of soil amendments (33) and to the amount of glucosinolate content in a crop (23). But few studies have been adapted for practical disease management by the growers, because of the difficulty in obtaining dry crucifer residue and the potential cost of application.

Since 1990, a sudden and widespread increase in Verticillium wilt of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) has been recorded. The disease, caused by *V. dahliae*, is an important production constraint in the Salinas Valley and other areas of coastal California. Extensive losses on crops harvested between April and October in infested fields have been recorded (21). In addition to cauliflower, the disease presents a significant long-term threat to other cool-season vegetable crops, because effective and economic control measures are currently unavailable. All the currently available commercial cauliflower cultivars are genetically very similar and susceptible to Verticillium wilt (21).

A detailed analysis of the host range of *V. dahliae* isolates obtained from artichoke, cabbage, cotton, pepper, potato, strawberry, tomato, and watermelon on cauliflower and the pathogenicity of two virulent isolates from cauliflower on all of the above crops, lettuce, and other crucifer crops indicated no host specificity associated with *V. dahliae* (36). However, isolates from cauliflower were only weakly pathogenic to broccoli and brussels sprouts and were not pathogenic to lettuce. Although cauliflower and broccoli have identical botanical names, they differ with respect to the nonoccurrence of Verticillium wilt on commercial broccoli crops (36) and lack of microsclerotial formation on broccoli roots.

A practical means of exploiting the beneficial effects of broccoli is to develop a rotational scheme with susceptible crops. It is, therefore, necessary to determine if fresh broccoli residue is as effective as dry broccoli and the temperature at which the benefits of broccoli are maximized; this information is useful in determining when to plant the crop and incorporate the residue into soil. The objectives of this study were to compare *V. dahliae* survival at different temperatures in unamended soil with survival in soils amended with fresh or dry residue, to determine the population dynamics of *V. dahliae* microsclerotia in soil over time as influenced by the three treatments at different temperatures, and to compare the effects of the three treatments on the growth of cauliflower and incidence of Verticillium wilt. Preliminary accounts of this work have been presented (37,38).

MATERIALS AND METHODS

Infested soil. Soil naturally infested with *V. dahliae* microsclerotia was collected from two cauliflower fields (top 15 to 20 cm) in the Salinas Valley, air-dried, and stored on benches in a greenhouse maintained at 25 ± 2°C. The soil from one field was a silty clay with a pH of 6.9 and 50% clay, 44% silt, 6% sand, and 3.3% organic matter. The soil from the other field was a sandy loam with a pH of 6.7 and 22% clay, 30% silt, 45% sand, and 2% organic matter. Subsequently, the soil was assayed for the number of microsclerotia using the modified Anderson sampler technique (1). Briefly, the soil was pulverized with a mortar and pestle and sifted through a 0.85-mm-pore-size sieve to remove soil clods. Ten grams of powdered soil was placed in screw-cap bottles and mixed with 2.5 ml of a 0.75% DL-methionine solution and incubated in the dark at 30°C for 1 week. The bottles were opened and allowed to air-dry for 1 week at 22 to 24°C. Samples were repulverized, and 0.5 g of soil was distributed onto six petri plates containing Sorensen's NP-10 selective medium using the modified Anderson sampler (1). Plates were incubated in the dark at 22 to 24°C for 3 weeks, and then the surfaces of the agar in plates were washed gently under running tap water to dislodge and remove soil particles. Washed plates were examined for clusters of *V. dahliae* microsclerotia using a stereomicroscope (10×), and the number of clusters from six plates were added and expressed as the number of microsclerotia per gram of soil. Populations of microsclerotia for each field were estimated twice to examine the variability in the technique.

Aliquots of 25 g of dry soil were sampled from the above field soil and added to wide-mouth plastic bottles (Nalgene, Rochester, NY). Treatments were randomly assigned to bottles as indicated below. Soil from one field was used in the first five experiments and from the other in four additional experiments to determine the effectiveness of treatments in different soil types.

Treatments and effects on *V. dahliae* microsclerotia in soil. To evaluate the effects of broccoli treatments on survival of microsclerotia in this study, three treatments including dry, fresh, and no broccoli amendments to *V. dahliae*-infested soil were tested at 10, 15, 20, 25, 30, and 35°C. To obtain dry broccoli for this study, fresh broccoli plants were collected prior to harvest from commercial fields, broccoli heads were separated and discarded (as very few broccoli heads remain in commercial fields after harvest), and the remaining plant chopped into fine pieces. This chopped broccoli residue was dried in an oven at 45°C for 2 days, ground into a fine powder, and stored at –10°C. The percentage of moisture in mature broccoli plants was determined gravimetrically. We had previously determined that an equivalent of 6 to 10% (wt/wt, approximate weight of broccoli residue relative to the soil weight in a hectare field) fresh broccoli residue remains in a commercial field after harvest, which is incorporated into the soil during the subsequent tillage operations. In all experiments, an equivalent to 8% fresh weight of broccoli residue was used.

Plastic bottles, each with 25 g of soil infested with *V. dahliae*, were randomly assigned to the three treatments and equivalents of 8% (wt/wt) fresh broccoli residue or the corresponding equivalent of dry broccoli (determined based on the amount of water in fresh broccoli) were added to six bottles for each experiment. Six bottles that did not receive any broccoli served as controls. The soil in each bottle was saturated to field capacity (to reflect the soil moisture conditions in commercial fields when the residue is incorporated), and one randomly chosen bottle of each treatment was assigned to incubators at 10, 15, 20, 25, 30, and 35°C. The soil was incubated for 45 days, and the number of *V. dahliae* microsclerotia from each treatment-temperature combination was determined as described above. The experiment was conducted nine times, with the assignment of temperature to an incubator being random each time.

The proportion of survival of *V. dahliae* microsclerotia in each treatment was computed by dividing the number of microsclerotia after 45 days of incubation by the number of microsclerotia at the beginning of the experiment. The soil used in experiments 1 to 5 contained 100 ± 4.6 microsclerotia per gram of soil, and that used in experiments 6 to 9 contained 82 ± 3.8 microsclerotia per gram of soil. Data on the proportion of survival were tested for normality, and no transformations were necessary to normalize the data. Experiments were considered as replications (blocks) for the analysis of variance conducted to determine the effects of treatment, incubation temperature, and treatment × temperature interactions. Both replications and treatments were considered as random effects in the analysis of variance. The sums of squares for temperature were further partitioned into linear, quadratic, and cubic trends to determine if the range of temperatures evaluated was appropriate. Because the treatment × temperature interaction was significant, there was strong evidence that the differences among treatments depended on temperature and, therefore, the treatments were compared using linear contrasts at each temperature (24). All analyses were performed using SAS (release 6.11 ed.; SAS Institute, Inc., Cary, NC).

To determine the population dynamics of *V. dahliae* microsclerotia as affected by the three treatments, experiments identical to the previous experiment were set up, and the soil was sampled three times at 15-day intervals and assayed for microsclerotia. This experiment was also conducted nine times, with the assignment of an incubator to a particular temperature being random each time.

Proportions of survival of *V. dahliae* microsclerotia at different sampling periods in each treatment-temperature combination were computed. Repeated measures analysis of variance was used to evaluate the effects of experiment (block), treatment, temperature, time, and interactions. The sums of squares for temperature were further partitioned into linear, quadratic, and cubic trends for each sampling period. The repeated measures analysis of variance showed that time interactions were not significant and, therefore, multivariate contrasts were made between treatments at each temperature, and Bonferroni adjustments (24) were made to the probabilities of contrasts for each sampling period.

Effects on growth and Verticillium wilt incidence on cauliflower plants. The effects of dry, fresh, and no broccoli amendments to soil were evaluated on the incidence of Verticillium wilt on cauliflower plants and on cauliflower growth attributes in the greenhouse. Experiments included both *V. dahliae*-infested field soil and autoclaved, pathogen-free field soil. Thus, there were a total of six treatments (dry, fresh, and no broccoli in two soil types) in each experiment. The number of *V. dahliae* microsclerotia was determined for each type of soil before the experiments were begun. Soil (both pathogen-infested and pathogen-free) was mixed with either fresh (8% wt/wt), dry (equivalent of 8% fresh broccoli determined based on the amount of water in fresh broccoli), or no broccoli. The amended soils were thoroughly mixed and 10 12-cm-diameter pots were filled for each treatment. The pots were arranged in a completely randomized design on a greenhouse bench (25 ± 2°C) and incubated for 2 weeks. Subsequently, a soil sample was removed from each pot within a treatment, bulked to obtain a composite sample for each treatment, and the number of *V. dahliae* microsclerotia was determined as described above. The susceptible cauliflower cultivar White Rock was subsequently direct-seeded in each pot and, after emergence, thinned to one plant per pot. All pots were fertilized once a week with Miracle Gro (15N-30P-15K; Scotts Miracle-Gro Products, Inc., Port Washington, NY) according to label directions. After 10 or 12 weeks of incubation in the greenhouse, soil from each pot within a treatment was sampled and bulked to determine the number of *V. dahliae* microsclerotia for each treatment. The plants were then washed free of soil, and data including height, number of leaves, and dry weights of shoot and root were collected for each plant. Foliar symptom severity and root discoloration severity were rated subjectively (36). Foliar symptom severity was rated on a scale of 0 to 4 in which 0 = normal (apparently healthy) plants; 1 = 25% of leaves showing chlorosis; 2 = 50% of leaves showing chlorosis; 3 = 51 to 74% of leaves showing chlorosis; and 4 = >75% of leaves showing chlorosis. Severity of root discoloration was rated on a scale of 1 to 4 in which 1 = normal (healthy) appearance; 2 = browning of <10% of lateral roots; 3 = browning of nearly 50% of lateral roots; and 4 = extensive browning of lateral roots and reduced lateral root system. Surface-sterilized stem segments were placed on acidified potato dextrose agar (APDA) to confirm *V. dahliae* infection. The APDA plates were incubated on laboratory benches for 3 weeks, and the number of plants yielding *V. dahliae* colonies was recorded. The experiment was conducted four times.

The means and standard errors of the mean for the number of *V. dahliae* microsclerotia for each treatment were calculated. Analysis of variance was conducted on all other variables to determine the overall effects of experiment, replication, treatment, and interactions. Means between treatments were compared using a least significant difference ($P = 0.05$) test.

RESULTS

Survival of *V. dahliae* microsclerotia at different temperature-treatment combinations. There were visible differences in the number of dark-colored fungal colonies other than *Verticillium* that grew on the NP-10 medium plated with soil from the three treatments. In general, plates with soil from the fresh broccoli treatment, regardless of the incubation temperature, contained far fewer numbers of the many contaminants observed on plates with soil from either the control or dry broccoli treatment. The number of contaminants on plates with the dry broccoli treatment was intermediate (data not shown). Regardless of the field from which the infested soil was collected, the effects of the treatments on fungi other than *Verticillium* were identical. Temperature differences between the incubation chamber and soil in vials were negligible.

Analysis of variance of the proportion of survival of the *V. dahliae* microsclerotia indicated that the results from the nine experiments were consistent and that there were no experiment × temperature and experiment × treatment interactions (Table 1). Survival of *V. dahliae* microsclerotia as affected by the treatments at different temperatures differed significantly and explained the greatest variation in the data. Both linear and quadratic terms for the temperature were highly significant ($P < 0.0001$). The temperature × treatment interaction was significant, indicating that the effectiveness of the treatments depended on the incubation temperature (Table 1).

TABLE 1. Summary analysis of variance for the proportion of reduction in *Verticillium dahliae* microsclerotia caused by dry, fresh, or no broccoli amendments to soil after 45 days of incubation at different temperatures

Source	df[y]	Sum of squares	Mean square	$P > F$[z]
Model	81	338,549.96	4,179.63	0.0001
Experiment (exp)	8	2,429.28	303.66	0.0748
Temperature (temp)	5	121,365.59	24,273.19	0.0001
Exp × temp ($error_a$)	40	7,570.30	189.26	0.2713
Treatment (trt)	2	178,414.75	89,207.38	0.0001
Linear	1	116,615.72	116,615.72	0.0001
Quadratic	1	4,517.81	4,517.81	0.0001
Exp × trt ($error_b$)	16	3,143.72	196.48	0.2750
Temp × trt	10	25,626.32	2,562.63	0.0001
$Error_c$	80	12,930.54	161.63	...

[y] Degrees of freedom.
[z] Probabilities associated with individual *F* tests.

The numbers of microsclerotia in the control were unaffected by temperatures up to 20°C during the 45-day incubation (Fig. 1). At 25°C, however, there was a small but significant ($P < 0.05$) increase over the initial populations. Higher temperatures by themselves (30 and 35°C) significantly reduced the numbers of microsclerotia in the control, 20% at 30°C compared with about 70% at 35°C (Fig. 1).

At each temperature, both dry and fresh broccoli treatments reduced the numbers of microsclerotia in soil significantly ($P \leq 0.0001$) compared with the no broccoli control (Table 2 and Fig. 1). At each temperature up to 30°C, fresh broccoli reduced the numbers of microsclerotia significantly ($P \leq 0.0001$) more than dry broccoli (Table 2). Differences between dry or fresh broccoli and control were greatest at 25 and 30°C. Differences between fresh and dry broccoli treatments were greatest at 20 and 25°C. At 35°C, however, differences between dry and fresh broccoli treatments were not significant (Table 2).

At both 10 and 15°C, fresh broccoli reduced the numbers of microsclerotia by nearly 60% during the 45-day incubation (Fig. 1). At higher temperatures, the reductions caused by fresh broccoli were incrementally higher. Above 25°C, the pathogen was nearly eliminated by fresh broccoli (Fig. 1). Even though dry broccoli consistently reduced the numbers of microsclerotia at each temperature, the reductions were not as high as in the fresh broccoli treatment (Fig. 1).

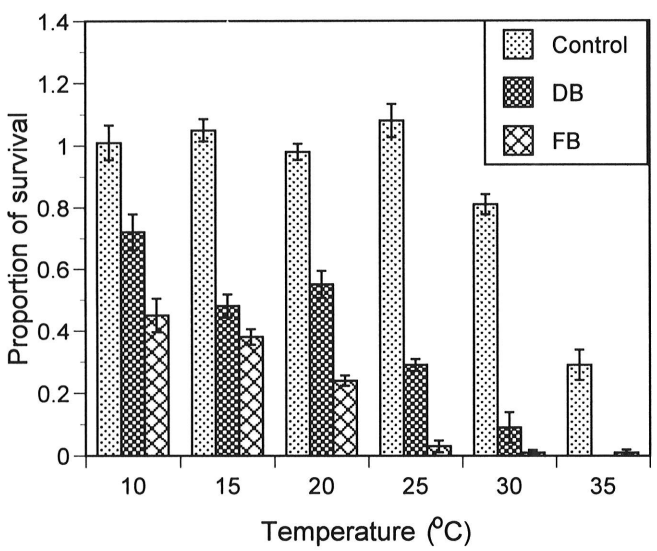

Fig. 1. Proportions of survival of *Verticillium dahliae* microsclerotia in naturally infested soils amended with dry (DB) or fresh (FB) broccoli residue or unamended (control) and incubated at different temperatures for 45 days.

Temporal dynamics of the broccoli-mediated reductions in microsclerotia. Repeated measures analysis of variance indicated that the time interactions with either the treatment or temperature were not significant. Data were, therefore, analyzed by individual sampling dates by the analysis of variance procedure. Across the three dates of sampling, results from all the experiments were consistent, and interactions with the experiment were not significant (Table 3). At each sampling time, the treatments explained the greatest variation in the data, followed by temperature and interactions between them. Both linear and quadratic terms for the effects of temperature were highly significant ($P < 0.0001$). Linear contrasts between control and the two broccoli treatments for each sampling time yielded results similar to the experiments with a single sampling (Table 2).

At each sampling time, the numbers of microsclerotia were significantly lower in both broccoli treatments than in the control. The magnitude of differences between broccoli treatments and

TABLE 2. Linear contrasts between the proportion of reduction in *Verticillium dahliae* microsclerotia caused by dry, fresh, and no broccoli (control) amendments at each temperature

Contrast	df[y]	Sum of squares	$P > F$[z]
Control vs dry broccoli at 10°C	1	2,964.50	0.0001
Control vs dry broccoli at 15°C	1	13,640.01	0.0001
Control vs dry broccoli at 20°C	1	7,854.22	0.0001
Control vs dry broccoli at 25°C	1	31,458.68	0.0001
Control vs dry broccoli at 30°C	1	32,342.72	0.0001
Control vs dry broccoli at 35°C	1	11,300.06	0.0001
Control vs dry broccoli (cumulative)	1	87,210.75	0.0001
Control vs fresh broccoli at 10°C	1	13,095.01	0.0001
Control vs fresh broccoli at 15°C	1	22,755.56	0.0001
Control vs fresh broccoli at 20°C	1	30,917.56	0.0001
Control vs fresh broccoli at 25°C	1	66,917.01	0.0001
Control vs fresh broccoli at 30°C	1	44,203.56	0.0001
Control vs fresh broccoli at 35°C	1	8,515.13	0.0001
Control vs fresh broccoli (cumulative)	1	167,442.19	0.0001
Dry vs fresh broccoli at 10°C	1	3,598.35	0.0001
Dry vs fresh broccoli at 15°C	1	1,160.01	0.0045
Dry vs fresh broccoli at 20°C	1	7,605.56	0.0001
Dry vs fresh broccoli at 25°C	1	6,612.50	0.0001
Dry vs fresh broccoli at 30°C	1	924.50	0.0096
Dry vs fresh broccoli at 35°C	1	196.68	0.1367
Dry vs fresh broccoli (cumulative)	1	12,969.19	0.0001

[y] Degrees of freedom.
[z] Probabilities associated with individual F tests. Bonferroni adjustments were made to the probabilities.

TABLE 3. Summary analysis of variance for the proportion of reduction in *Verticillium dahliae* microsclerotia caused by dry, fresh, or no broccoli amendments to soil after 15, 30, and 45 days of incubation at different temperatures

		Sampling time					
		15 days		30 days		45 days	
Source	df[x]	MS[y]	$P > F$[z]	MS	$P > F$	MS	$P > F$
Model	81	4,013.93	0.0001	4,086.16	0.0001	4,084.04	0.0001
Experiment (exp)	8	460.08	0.2672	455.72	0.1289	1.64	0.1277
Temperature (temp)	5	15,006.21	0.0001	20,626.40	0.0001	19,811.25	0.0001
Linear	1	66,453.36	0.0001	97,574.81	0.0001	90,909.88	0.0001
Quadratic	1	8,487.72	0.0001	4740.67	0.0001	7,205.38	0.0001
Exp × temp ($error_a$)	40	365.09	0.4684	254.12	0.6223	330.05	0.2647
Treatment (trt)	2	108,931.80	0.0001	100,283.17	0.0001	98,857.42	0.0001
Exp × trt ($error_b$)	16	358.12	0.4716	230.20	0.6550	275.32	0.4841
Temp × trt	10	821.93	0.0208	978.71	0.0007	1,275.93	0.0001
$Error_c$	80	360.24	...	279.33	...	280.36	...

[x] Degrees of freedom.
[y] Mean square.
[z] Probabilities associated with individual F tests.

control were greatest for fresh broccoli at each temperature and sampling time. Greatest reductions in the numbers of microsclerotia occurred within 15 days of incubation in both broccoli treatments at each temperature. Changes at subsequent sampling times were not significant (Fig. 2).

Treatment effects on Verticillium wilt incidence on cauliflower plants and their growth. Populations of *V. dahliae* microsclerotia decreased significantly (50 to 60%) in both dry and fresh broccoli-amended, infested soil in pots within 2 weeks. Numbers of microsclerotia in unamended soil remained either the same or registered a marginal increase by the conclusion of each experiment. In autoclaved soil, there were no microsclerotia in any treatment in three of four experiments (Table 4).

In general, plants in broccoli-amended pots were more robust than in the unamended controls, despite the biweekly fertilization of all plants. Plants in both broccoli treatments in infested soil were significantly taller and had greater root and shoot dry weights than in unamended controls. Differences in plant heights between the two broccoli treatments were not significant for either infested or autoclaved field soil (Table 5). The number of leaves on plants in the different treatments were nearly identical, even though leaves in the broccoli-amended treatments were visibly broader and greener. The root and shoot dry weights of plants in broccoli-amended pots with infested soil were significantly greater than in all other treatments (Table 5). Broccoli amendments to autoclaved soil did not result in improved shoot and root dry weights of cauliflower plants. The root discoloration, severity index, and wilt incidence were all significantly lower in plants in infested soil from broccoli-amended pots than in unamended controls (Table 5). Differences between dry and fresh broccoli were significant only for wilt incidence; wilt incidence in plants from fresh broccoli-amended soil was significantly lower (Table 5).

DISCUSSION

Broccoli residue reduced the number of *V. dahliae* microsclerotia in soil regardless of the temperature, but the magnitude of reduction depended on the temperature. Fresh broccoli residue was considerably more effective than dry broccoli in suppressing *V. dahliae* microsclerotia. This information can be utilized in a Verticillium wilt management program by developing a rotational scheme of susceptible crops with broccoli and incorporating the residue after broccoli harvest when the temperature is at least 20°C. In the Salinas and adjoining coastal valleys in California, this entails planting broccoli as a winter crop between November to January and, after commercial harvest, incorporating the residue during April to June. The practice would be compatible with the current production practices in the area and could be easily adapted by the growers to maximize the broccoli-mediated pathogen suppression.

Soil amendments with each of nine crucifer species reduced the severity of cabbage yellows and populations of *Fusarium oxysporum* f. sp. *conglutinans* (33). All crucifer species tested reduced the severity of cabbage yellows equally well. However, the effec-

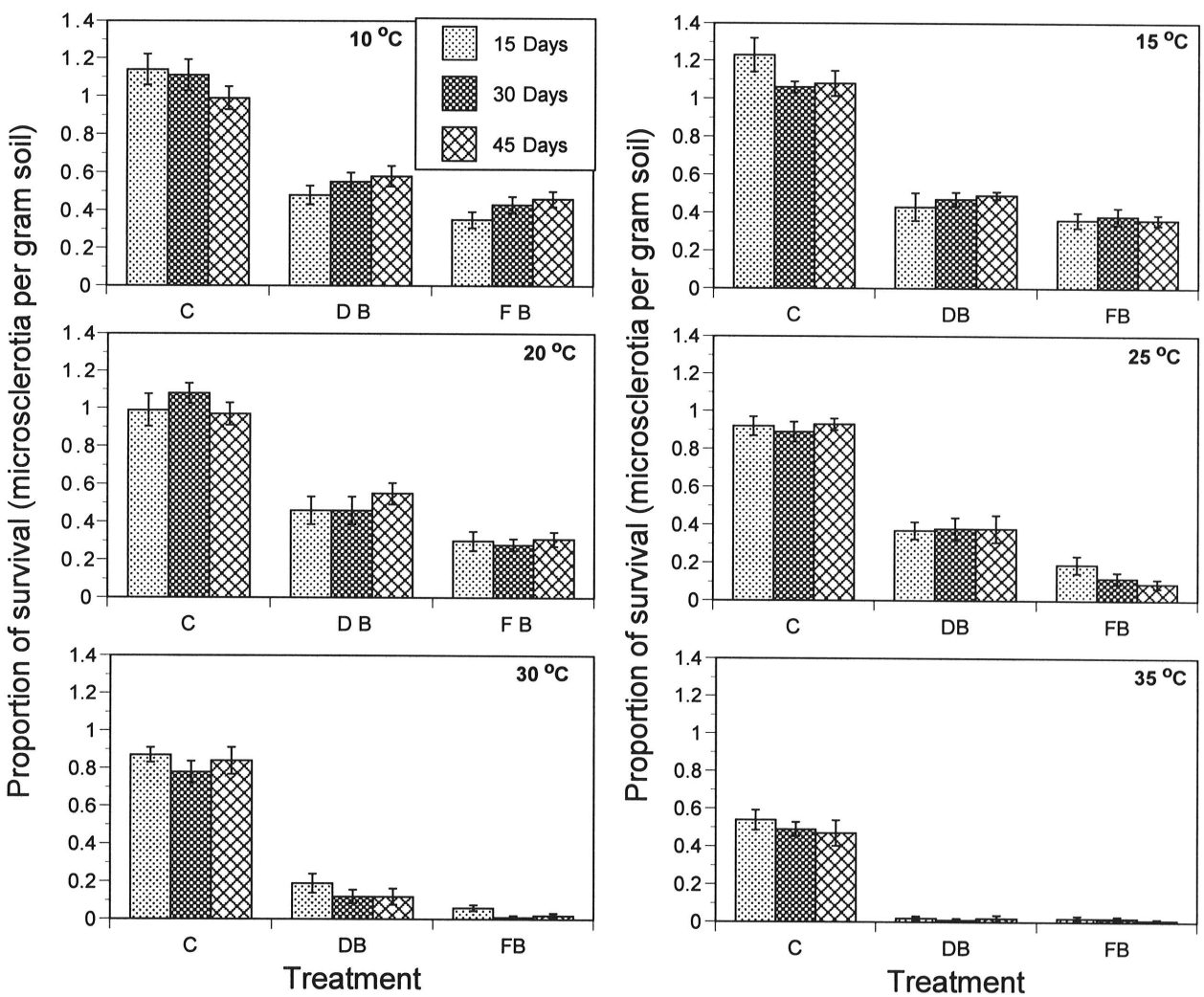

Fig. 2. Proportions of survival of *Verticillium dahliae* microsclerotia at intervals of 15 days in naturally infested soils amended with dry (DB) or fresh (FB) broccoli residue or unamended (control) and incubated at different temperatures.

tiveness of the amendments was dependent on dehydration of crucifer residues before incorporation into soil, their concentration, and time of exposure (33). This is in contrast to the results obtained in this study, albeit the crucifer species used in the two studies were different. Also, the glucosinolate hydrolytic products are very likely to be different in fresh crucifer tissue compared with the dry material (9). Even though the authors (33) observed that dehydrated crucifer residue can be stored and applied by the growers as required, factors such as the enormous amounts of dried material needed to obtain the desired results, increased costs of drying the material and its application, and increased energy use clearly discourage its use. In this study, however, regardless of the temperature, fresh broccoli was more effective than dry broccoli and the effects were significantly greater between 20 and 30°C. In addition to reduced numbers of *V. dahliae* microsclerotia in soil, fewer dark fungal colonies on highly selective NP-10 medium were observed in plates with soil amended with fresh broccoli, regardless of the incubation temperature. These results may encourage the adaptation of rotations of broccoli with the corresponding crop at least in the coastal valleys of California.

The mechanisms by which crucifer residues act on plant pathogens are assumed to be mostly chemical. Most of the previous studies have tested the effectiveness of crucifer residue or of total or specific gases emanating from the residues on nonsclerotial fungal pathogens. Because most glucosinolate breakdown products are volatile (9,10,11,13), their retention in the soil environment is very short. Sclerotial fungi, however, survive in soil despite adverse weather conditions for prolonged periods of time (14,16,34). Thus, a transient exposure to the volatile gases may be insufficient to affect the viability of a significant number of *V. dahliae* microsclerotia. As the microsclerotia may be located both in the soil and in the organic debris, they may not be uniformly exposed to the volatile gases. It is quite possible that other biological mechanisms in broccoli residue-amended soil operate in affecting pathogen propagule survival. A number of other soil amendments have been tested against *V. dahliae* with mixed success (8,19,27), but none have been adapted for Verticillium wilt management, because of the drawbacks of using dried plant material discussed above.

In a previous study on the effectiveness of soil solarization, nearly 90% of *V. dahliae* microsclerotia were killed within 29 days at 37°C (31). This is consistent with results obtained at 35°C in this study. In experiments with only one soil sampling after 45 days of incubation, nearly 70% of the microsclerotia did not survive incubation at 35°C even in the control (no broccoli) treatment. In experiments with sampling at 15, 30, and 45 days, nearly 50% of microsclerotia were destroyed at 35°C within 15 days in the control treatment. Additionally, the propagules that survived at 30 and 35°C may have been weakened (39), and the combination of broccoli and temperatures above 25°C increased the mortality of the propagules. The effects of temperature in experiments with single or multiple sampling were quadratic and, thus, testing temperatures above 35°C would not have provided any additional information. In the coastal valleys in California, however, soil temperatures above 25°C seldom occur and, thus, propagules are not affected by temperature alone. Hence, rotations with broccoli would provide an alternative means of managing this pathogen. During the past 3 years, we have demonstrated the effectiveness of this approach both in experimental and grower fields (K. V. Subbarao, *unpublished data*). The temperatures at which broccoli residue had the greatest effect above and beyond that of temperature alone were also the optima for growth of a number of *V. dahliae* strains from various hosts on agar media (36).

Since the distribution of microsclerotia in commercial field soils varies from uniform to aggregated, it is important to incorporate broccoli residue uniformly in the field. Growers in the Salinas Valley currently use incorporation methods that provide a uniform distribution of plant residues in their fields. The key for an effective Verticillium wilt management in these fields, however, is to develop rotations with crops such as broccoli. Most reductions in the microsclerotia in soil caused by broccoli treatments occurred after 15 days of incubation. Since we did not sample soil before 15 days of incubation, it is unclear specifically when the reductions occur. In commercial fields, how-

TABLE 4. *Verticillium dahliae* microsclerotia in infested (I) and autoclaved (A) soil amended with dry, fresh, or no broccoli at two sampling times in four greenhouse experiments (exp.) to test the effects of these amendments on the growth and Verticillium wilt in cauliflower plants

	Mean microsclerotia (± standard errors of the mean) per gram of soil[z]							
	2 weeks after amendment				10 to 12 weeks after amendment			
Treatment	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4
I – broccoli	22 ± 2.0	39 ± 3.5	7 ± 1.3	23 ± 0.7	24 ± 3.1	40 ± 2.3	8 ± 1.2	28 ± 3.5
I + dry broccoli	13 ± 3.5	8 ± 5.3	1 ± 0.7	3 ± 0.7	17 ± 2.9	8 ± 4.6	0 ± 0.0	9 ± 1.8
I + fresh broccoli	15 ± 0.7	6 ± 1.2	0 ± 0.0	0 ± 0.0	15 ± 0.7	5 ± 0.7	0 ± 0.0	3 ± 0.7
A – broccoli	2 ± 1.2	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	1 ± 0.7
A + dry broccoli	2 ± 1.2	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 1.2	0 ± 0.0	0 ± 0.0	0 ± 0.0
A + fresh broccoli	1 ± 0.7	0 ± 0.0	0 ± 0.0	0 ± 0.0	5 ± 0.7	0 ± 0.0	0 ± 0.0	0 ± 0.0

[z] The initial densities of *V. dahliae* microsclerotia in soil were 22.0, 22.0, 7.0, and 22.0 per gram of soil for experiments 1, 2, 3, and 4, respectively, for infested soil, and 0.0 microsclerotia per gram of soil for all experiments for autoclaved soil.

TABLE 5. Verticillium wilt and cauliflower growth in *Verticillium dahliae*-infested (I) and autoclaved soil (A) amended with dry, fresh, or no broccoli

Treatment	Plant height (cm)	Number of leaves	Dry weight (g)		Severity index[w]	Root discoloration[x]	Wilt incidence (%)[y]
			Shoot	Root			
I – broccoli	18.0 b[z]	8.4 b	3.48 b	0.43 bc	2.4 a	3.1 a	83.0 a
I + dry broccoli	22.3 a	8.6 ab	5.75 a	0.81 a	0.6 c	1.8 bc	52.0 b
I + fresh broccoli	22.9 a	9.1 a	5.95 a	0.75 a	0.4 c	1.5 c	37.0 c
A – broccoli	18.5 b	8.6 ab	3.90 b	0.57 bc	1.2 b	1.9 b	1.0 d
A + dry broccoli	22.4 a	8.4 b	3.64 b	0.58 b	0.4 c	1.6 c	0.0 e
A + fresh broccoli	22.0 a	8.2 b	3.32 b	0.42 c	0.4 c	1.4 c	0.0 e

[w] Recorded on a 0 to 4 scale, in which 0 = normal plants and 4 = >75% leaves showing chlorosis.
[x] Symptoms on roots recorded on a 1 to 4 scale, in which 1 = normal appearance and 4 = extensive browning and reduced root laterals.
[y] Percentage cauliflower plants showing Verticillium wilt symptoms.
[z] Values followed by the same letter within each column are not significantly different according to a least significant difference test ($P < 0.05$). All variables are means of 10 plants each in four experiments.

ever, the period between crop residue incorporation and planting of the next crop requires more than 2 weeks in the Salinas Valley. Thus, the specific sequence timing of reduction after the incorporation of residue appears to be less important from a practical perspective.

Although the present study demonstrated the effects of broccoli on *V. dahliae* microsclerotia from cauliflower fields, it is quite likely that similar effects are possible on soil populations of microsclerotia from other Verticillium wilt susceptible crops. However, additional field studies are required for adaptation of this finding into other cropping systems.

In addition to the reduction in soil populations of microsclerotia, broccoli residue also resulted in enhanced growth of cauliflower plants in amended soil. The significant growth increases in cauliflower plants recorded in this study may have been caused by a combination of increased root growth and enhanced nutrient uptake, as addition of crop residue may make available increased nitrogen in the soil (29). In our study, the effect of additional nitrogen may have been obscured, as all plants were fertilized with additional nitrogen at biweekly intervals. It is possible that the residue alters the soil microflora that in turn enhance the growth of crop plants. Although plant height was equally enhanced in autoclaved and nonautoclaved field soil, plant dry weights were significantly higher in broccoli-amended as compared with nonamended only in nonautoclaved soils.

In summary, broccoli appears to be a versatile crop in that it is a vegetable with medicinal value (30,44) and also has deleterious effects on many plant pathogens. It may become an increasingly important crop for the management of plant diseases as chemical fumigants are withdrawn from use as a result of their effect on the environment.

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