Control of Cabbage Yellows (Fusarium oxysporum f. sp. conglutinans) by Solar Heating of Field Soils Amended with Dry Cabbage Residues

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ABSTRACT

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Population counts of Fusarium oxysporum f. sp. conglutinans were greatly reduced and cabbage yellows was undetected in plots treated with solar heating of soils and cabbage amendments (1%, w/w). Dried cabbage was mixed in soil and covered with a translucent polyethylene tarp (solar heating) for 4 or 6 wk. Both solar heating alone and cabbage amendments plus cover under shade were effective but not as effective as the combination of solar heating and cabbage amendments. In contrast, cabbage amendments, not covered, either under shade or direct sunlight were ineffective. It is suggested that a tarp is necessary not only to increase the temperature of the soil to critical levels under solar heating but also to trap fungitoxic gases emanating from the cabbage amendments.

A new race, race 5, of Fusarium oxysporum f. sp. conglutinans (Wr.) Snyd. & Hans. that is capable of causing disease in cabbage (Brassica oleracea var. capitata L.) with type A monogenic resistance was previously reported (11). Because most of the Fusarium yellowsresistant cultivars are highly susceptible to race 5 (11), we studied a new approach for controlling soilborne pathogens and weeds by solar heating of soil amended with organic residues. Soil fumigation, though potentially effective for controlling many soilborne plant pathogens, probably is not economically feasible for cabbage.

Incorporation of dried cruciferous amendments to solar-heated soil in pots buried in the field nearly eliminated F. o. f. sp. conglutinans, and in the laboratory, toxic volatile compounds from decomposing cabbage either directly or indirectly eliminated F. o. f. sp. conglutinans from soil (J. Ramirez-Villapudua and D. E. Munnecke, unpublished). The purpose of this study was to attempt to reduce or eliminate F. o. f. sp. conglutinans in the field and thus to control cabbage yellows by combining solar heating with cabbage residue soil amendments.

MATERIALS AND METHODS

Infestation of a field with F. o. f. sp. conglutinans. A field plot of sandy loam

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soil at the University of California, Riverside (UCR), was used. The field (13 × 25 m) was plowed, rototilled, and fumigated with methyl bromide (400 lb/acre) to facilitate the establishment of the fungus. F. o. f. sp. conglutinans was not detected on Komada's medium (5) from random soil samples from depths of 0-20 cm collected before and after fumigation. Seeds of the susceptible cabbage cultivar Rio Verde were planted in pots of the soil and kept in a growth chamber 24 and 28 C (day/night temperatures) for 5 wk. No colonies of F. o. f. sp. conglutinans were recovered from the soil or yellows-affected plants found.

Two weeks after fumigation, the plot was infested with F. o. f. sp. conglutinans race 5 by transplanting 20-day-old Rio Verde cabbage seedlings dipped in a spore suspension containing 1×10^6 macroconidia per milliliter of F. o. f. sp. conglutinans at 10-cm intervals in the field in furrows 30 cm apart. Severe symptoms of cabbage yellows occurred in all plants examined within 30 days of inoculation. The field additionally was infested after the appearance of symptoms of the disease on the transplants by dispersing 150 kg of wheat bran-sand (15) cultures of F. o. f. sp. conglutinans uniformly over the field. The diseased plants and the bran inoculum were plowed and rototilled to incorporate them into the soil, and the plot was sprinkler-irrigated 1 hr daily for 2 wk. The soil was sampled at intervals from 0 to 40 cm deep, and it was found to be evenly infested with the fungus.

Preparation of wheat bran-sand cultures of F. o. f. sp. conglutinans. Wheat bran was prepared by grinding wheat seed in a Wiley mill and passing it through an 85-mm (20-mesh) sieve. A bran-sand medium was prepared by mixing the following: wheat bran, 200 g; white silica sand, 500 g; and distilled water, 500 ml. Wheat bran-sand medium (4-kg portion) was dispensed into large heat-resistant plastic bags. The open ends of the plastic bags were gathered around a large cotton plug (7 cm diameter) in which a glass tube (1.8 cm diameter) with an exchangeable cotton plug had been inserted. The bag, cotton, and tube were tied securely to make a tight fit. After autoclaving for 2 hr, the medium was infested with 500 ml of conidial suspension ($10 \times 10^6/\text{ml}$) of a 5-day-old culture of F. o. f. sp. conglutinans. The suspension was added to the medium by removing the small plug from the glass tube and pouring the suspension in the bag. After inoculation, the cotton was aseptically replaced in the glass tubes, the medium thoroughly mixed, and the bags inflated by blowing air by mouth into them. If the bags were not inflated, very sparse growth of F. o. f. sp. conglutinans was obtained. No contamination by other organisms was observed. After 20 days at room temperature, the medium and fungus (150 kg dry weight) were thoroughly mixed, placed on a table, and a clear polyethylene sheet was suspended 20 cm above the mixture to conserve moisture and allow continued growth of the fungus for 1 wk. No precautions were taken to prevent introduction of other organisms. The sheet was removed and cultures were air-dried for 5 days, ground in a Wiley mill, and passed through a 20-mesh screen, then the mixture of conidia, mycelia, and chlamydospores was dispersed over the field.

Preparation of cabbage residues. In 1983, about 3,000 heads of the cabbage cultivar Headstart (susceptible to race 5 of F. o. f. sp. conglutinans) were chopped with a machine and dried in the field for 10 days. The residue was raked, collected, and bagged. In 1984, about 4,000 heads of cabbage were chopped by machine into approximately 4-cm-diameter pieces and scattered on a plastic sheet to dry. The chopped cabbage was stirred daily with a side-delivery rake on a tractor to speed drying. After 10 days, the sun-dried cabbage was used in the plot.

Field plot design. One experiment was conducted from 5 September to 23 October 1983, and another was done on

the same site from 9 July to 9 August 1984 at UCR. The field was fallowed between experiments. Each treatment was replicated three times in a randomized block design (Fig. 1). Plots were 5.7×2.4 m in 1983 and 2.5×3.5 m in 1984. The cabbage amendment, applied at the rate of 1% (estimated) by weight of soil in both experiments, was mixed to a depth of about 15 cm in the soil with a Rototiller. The soil was irrigated by sprinklers to a depth of 80 cm 3 days before application of the translucent plastic covers. Plots treated with solar heating were covered with polyethylene, about 50 µm thick, specially formulated to withstand solar irradiation. Uncovered plots were sprinkled at 3-day intervals to retain soil moisture. The following treatments were applied for the 1983 experiment, and all of them were exposed to full sunlight: solar heating plus cabbage amendment; cabbage amendment, no solar heating; no cabbage amendment, solar heating; and no cabbage amendment, no solar heating. The second 1984 test was done in the same field. Four treatments were exposed to solar radiation as in the first trial, and four were shaded. Shading was provided by enclosing the plots under black polyethylene tents so that air

circulated freely beneath them (Fig. 1). The shaded treatments were as follows: cabbage amendment, covered; cabbage amendment, not covered; no cabbage amendment, covered; and no cabbage amendment, not covered.

Cross-contamination of plots was minimized by fencing, by ditching between plots to prevent waterborne contamination, and by wearing clean plastic bags over shoes while working in the plots. New shoe bags were used for each plot. The plots remained weedfree until the end of the treatment period without using herbicides.

Temperature records. Soil temperatures in covered and uncovered plots were continuously recorded with thermographs from sensors buried at several depths (Table 1).

Assay of effectiveness of treatments. Soil samples were taken from the 15-cm depth at the end of the 45-day (1983) treatment period. Cabbage seeds of Rio Verde were sown in these samples of soil and grown in a growth chamber at 24-28 C for 30 days. Disease severity was determined on individual plants according to the rating system devised by Williams (14). The data were expressed as the means of three replicate samples per treatment, with a total of 40 seedlings per sample.

The population density of F. o. f. sp. conglutinans as well as the disease severity of potted test plants planted in samples of soil was determined in soil samples taken at the end of the 30-day experiment. Five samples (250 g each) from each plot collected at depths of 0-10, 10-20, 20-30, and 30-40 cm were

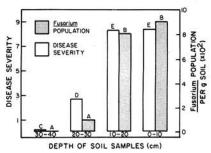


Fig. 2. Vertical distribution in soil of propagules of Fusarium oxysporum f. sp. conglutinans in the field at the beginning of the experiment in 1983. Also indicated are disease severity ratings of cabbage seedlings (cultivar Rio Verde) planted in corresponding soil samples grown in a growth chamber at 28 C (day) and 24 C (night). Letters A and B refer to statistical significance of populations of F.o.f. sp. conglutinans; C-E refer to statistical significance of disease severity. The same letter above bars indicates that the means (three replicates) of the data are not significantly different (P = 0.05) according to Duncan's multiple range test.



Fig. 1. Field plot in 1984. Half of the treatments were exposed to solar radiation and half were shaded by enclosing them under black polyethylene tents.

Table 1. Highest (H) and mean of the daily maximum (M) soil temperatures attained in soil in field plots at University of California, Riverside

	Soil depth and temperatures (C) attained ^a									
	10 cm		20 cm		30 cm		40 cm			
Treatment	н	M	н	M	н	M	н	M		
5 Sept20 Oct. 1983										
Full sun										
Tarped	53.3	42.4	39.4	32.0	35.1	25.5	34.8	26.3		
Not tarped	37.6	33.6	NA ^b	NA	NA	NA	NA	NA		
9 Jul9 Aug. 1984										
Full sun										
Tarped	51.7	49.6	38.3	35.8	38.3	37.3	38.0	37.0		
Not tarped	40.3	36.4	36.1	33.1	35.1	33.1	34.4	31.7		
Shaded										
Tarped	32.8	30.9	NA	NA	33.0	30.1	33.0	29.3		
Not tarped	31.9	31.2	NA	NA	33.1	30.3	32.7	29.7		

^a Temperatures recorded at 2:00 P.M. with a soil thermograph.

^bNot available.

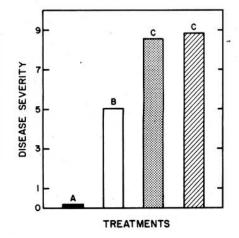




Fig. 3. Effects of solar heating and amendments of 1% dried cabbage (w/w) on severity of cabbage yellows in soil samples collected at a depth of 15 cm (field plot in 1983), sampled 45 days after treatment. The same letter above bars indicates that the means (three replicates) of the data are not significantly different (P=0.05) according to Duncan's multiple range test.

bulked for each 10-cm interval within a plot and mixed thoroughly in plastic bags. The air-dried soil (100 g) from each depth was diluted in 1,000 ml of sterile water and shaken vigorously for 30 min. Serial dilutions to 1×10^{-3} of the original suspension were made. Samples (0.5-ml)

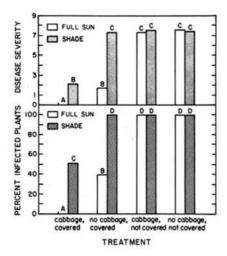


Fig. 4. (Top) Disease severity of Rio Verde cabbage plants and (bottom) percent infected plants 70 days after seeding in the field plot in 1984. The same letter above bars indicates that the means (three replicates) of the data are not significantly different (P=0.05) according to Duncan's multiple range test.

were spread uniformly on plastic petri dishes containing 15 ml of Komada's medium (5). Five petri plates were prepared for each dilution. $F.\ o.\ f.\ sp.\ conglutinans$ colonies, which could be identified visually, were counted after incubation for 8 days at 25 ± 2 C under diffuse light.

Seeds of Rio Verde (susceptible to races 1 and 5 of F. o. f. sp. conglutinans) cabbage were sown in soil taken from the field at the various depths. After emergence, the seedlings were thinned to 20 per pot and grown for 5 wk at 28 and 24 C (day/night) in a growth chamber. Disease severity was rated (14) on 60 seedlings per sample by averaging the data from three replicates per treatment.

Field plots were hand-seeded densely with the Rio Verde cultivar in five rows per plot, about 40 cm apart. After emergence, cabbage seedlings were thinned to 5-cm intervals. Plants were observed frequently for yellows symptoms, and after 70 days, disease severity ratings were made from the three central rows. Disease incidence also was evaluated by selecting 50 plants at random from each plot and rating them on the basis of presence or absence of xylem discoloration. Finally, roots were excised, washed in tap water, immersed in 0.5% sodium hypochlorite for 2 min, cut into segments about 0.5 cm long, and plated on Komada's medium.

RESULTS

Vertical distribution of F. o. f. sp. conglutinans in the field. At the start of the experiments, F. o. f. sp. conglutinans propagules per gram (p/g) in the infested soil were as follows: 1-10 cm, 9.1×10^2 p/g; 10-20 cm, 8.2×10^2 p/g; 20-30 cm, 1 \times 10² p/g; and 30-40 cm, 0 p/g (Fig. 2). Soil samples from the four depths were planted with seeds of Rio Verde cabbage, and the disease was assayed daily after emergence for 35 days. Most of the plants were killed in the series 0-10 and 10-20 cm (disease rating of about 8.3). The p/g count at 20-30 cm did not differ significantly from the 0 count obtained at 30-40 cm, yet the disease ratings between the two depths were significantly different.

The effects of solar heating and amendments of dried cabbage (1%, w/w) on severity of cabbage yellows on plants in soil collected at a depth of 15 cm were determined by growing cabbage seedlings in the soil for 5 wk in a growth chamber. Almost complete control of cabbage yellows was obtained by use of solar heating plus cabbage amendments. The disease was only partially controlled by solar heating alone (Fig. 3). For example, disease severity after solar heating plus cabbage amendments or solar heating alone was 0.2 or 5.0, respectively. In contrast, disease severity ratings were 8.8 in soil treated with cabbage (no cover)

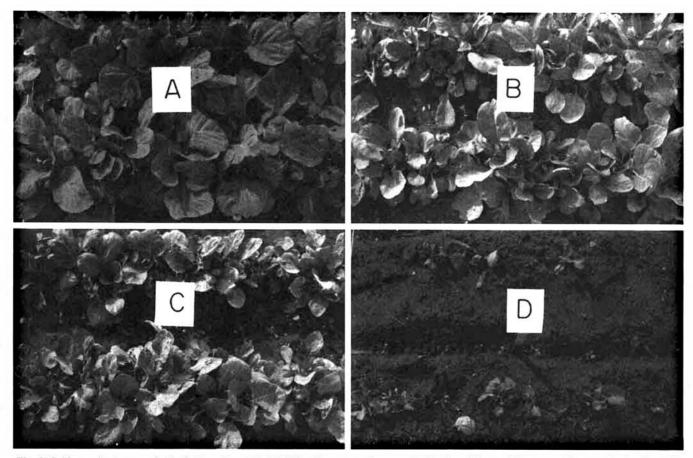


Fig. 5. Cabbage plants grown in the field and treated with (A) cabbage amendment, solar heating; (B) no cabbage amendment, solar heating; (C) cabbage amendment, shaded; and (D) cabbage amendment, not covered, shaded.

and 8.5 in the completely untreated control plot (no cabbage, no cover).

Ratings made in the field on the incidence and severity of disease confirmed that solar heating plus cabbage amendments, solar heating alone, or cabbage amendments with cover under shade, in decreasing order of control, significantly reduced cabbage yellows (Figs. 4 and 5). Of particular interest were the results obtained by solar heating plus cabbage amendments, because no diseased plants were found

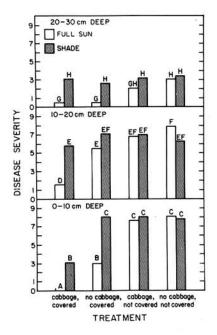


Fig. 6. Disease severity of Rio Verde cabbage seedlings 5 wk after planting in samples of soil taken in 1984 from the field at various depths. The same letter above bars indicates that the means (three replicates) of the data are not significantly different (P = 0.01) according to Duncan's multiple range test.

even 70 days after seeding.

Disease severity ratings of plantings in the plots indicated that solar heating alone and cabbage amendments, covered and shaded, were not significantly different (P = 0.05), but there was a difference in incidence of disease between plantings in the two treatments (40 and 50%, respectively). The other treatments were as ineffective as the control (no cabbage, not covered, either in shade or full sunlight), and no significant differences between them were found. In these treatments, high percentages of plants were infected, disease severity ratings were high, and a large number of plants were dead.

Cabbage yellows severity and percentage of infected plants were reduced in plantings in plots covered and amended with cabbage, either under shade or exposed to sunlight, whereas no control of the disease was obtained when plots were amended with cabbage but not covered. Because soil temperatures were increased by the covers, the resulting control of cabbage yellows was outstanding.

Disease severity evaluations made in the greenhouse (Fig. 6) on samples of soil taken from the field confirmed results obtained in the first experiment: Fusarium yellows was markedly reduced by solar heating plus cabbage residue and moderately reduced by both solar heating alone or by cabbage residue, tarped and shaded (Fig. 6). Severity of cabbage yellows on cultivar Rio Verde was significantly reduced (P = 0.01) in plantings of soil from all depths by solar heating plus cabbage amendments. No diseased plants were observed in soil samples taken from the upper 10-cm layer, and the suppressive effect was carried through the depths of 0-30 cm. Cabbage amendment, covered and shaded, was almost as effective as solar heating alone. Solar heating significantly reduced disease severity at the upper 10-cm layer, but its effect was considerably less at 10-20 cm. Of particular interest was the control of cabbage yellows at 20-30 cm by solar heating. In contrast, the highest disease severity ratings were recorded in the remaining treatments, which were not significantly different from the controls (no cabbage amendment, not covered, either in sunlight or shade).

Fungal population counts indicated that F. o. f. sp. conglutinans propagules were markedly reduced by solar heating plus cabbage residue at all depths. Again, both solar heating alone and cabbage amendments plus cover under shade reduced populations of F. o. f. sp. conglutinans but not as much as the combination of solar heating and cabbage amendments (Table 2). The highest population reduction was recorded in the upper 10-cm layer, coinciding with the highest temperature readings (Table 1). These results indicate that control of F. o. f. sp. conglutinans populations may be correlated with the high temperatures attained in the upper layers of the soil and probably with the cabbage volatiles trapped beneath the solar tarp.

Excellent control of weeds was obtained by three treatments. No weeds were observed for several months in soils treated with solar heating plus cabbage amendments; a few weeds, mainly Amaranthus sp. and some grass weeds, grew in soils treated with solar heating alone or with cabbage amendments, covered and shaded. In contrast, weed growth was prolific in the other soils.

DISCUSSION

These results support the hypothesis of Katan (3) that solar heating may be

Table 2. Effects of cabbage amendments, solar radiation, and shade treatments after 30 days on propagules of Fusarium oxysporum f. sp. conglutinans race 5 per gram of soil (p/g) at various depths

Environment and depth in soil (cm)	Treatment										
	Cabbage amendment, covered ^y		No cabbage amendment, covered		Cabbage amendment, not covered		No cabbage amendment, not covered				
	p/g	Reduction (%)	p/g	Reduction (%)	p/g	Reduction (%)	p/g	Reduction (%)			
Full sunlight		100 7		0.7			705				
0-10	0	100 a ²	27	97 a	650	17 b	785	0 ь			
10-20	44	94 a	122	84 a	644	15 Ъ	760	0 Ь			
20-30	40	. 72 a	55	69 a	66	54 a	143	0 ь			
Mean	28	95 a	68	88 a	453	19 Ь	563	0 Ь			
Shaded			1								
0-10	66	92 a	775	1 b	660	16 b	777	1 b			
10-20	111	85 a	670	12 b	666	12 b	655	14 b			
20-30	90	37 ab	135	−6 b	148	−4 b	145	-1 b			
Mean	89	84 a	527	6 b	491	12 b	525	7 Ь			

Surface of soil tightly covered with polyethylene tarp.

Percentage of population reduction for each soil depth was calculated as $(1 - A/B) \times 100$, where A = p/g for a treatment and B = p/g from control (no cabbage, not covered, under full sunlight). In each soil depth, values followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

enhanced by addition of suitable organic residues to the soil. In our research, F. o. f. sp. conglutinans was practically eliminated and cabbage yellows was undetected in the field by combining solar heating with dried cabbage amendments. In contrast, cabbage amendments not covered, either in shade or sunlight, were ineffective. Thus, the use of a tarp is necessary for optimum success of control.

The assumption that gases from decomposing cabbage residues are involved in the control of cabbage yellows in the field is supported by laboratory data (unpublished) where fungitoxic gases were detected from cabbage-amended soil and cabbage without soil. Mycelial growth, sporulation, and propagules of F. o. f. sp. conglutinans in soil were markedly reduced in closed containers, but propagules of F. o. f. sp. conglutinans in soil were stimulated in open jars. The degree of fungal growth was directly related to the cabbage residue concentrations and time of exposure.

Fungitoxic gases from decomposing cabbage were reported by Lewis and Papavizas (6-8), who demonstrated the toxicity of sulfur-containing volatile substances (7) and ammonia (8) on Aphanomyces euteiches and Rhizoctonia solani. These findings may explain the controversial results of Zakaria and Lockwood (15), who demonstrated that soybean meal amendments enclosed in containers effectively controlled Fusarium spp. in the laboratory but not in the field, probably because the plots were not covered and the gases, if produced, may have escaped. We obtained results with cabbage amendments that suggested that toxic volatile compounds probably were trapped beneath the plastic tarp and promoted the decline of propagules of F. o. f. sp. conglutinans. Permeability of polyethylene tarps to most gases is low (2); therefore, it is possible that volatile compounds from cabbage amendments accumulated beneath the plastic tarp in amounts high enough to decrease the pathogen populations.

The effectiveness of solar heating in the upper soil layer was related to the higher soil temperatures attained in that layer

(Tables 1 and 2). The control attained by solar heating alone corresponded to the maximum soil temperatures attained (Table 1). Maximum temperatures during the treatment period were lower in 1983 than in 1984, and correspondingly, the effect of solar heating on disease severity was less in 1983. Because the highest soil temperatures were recorded in the upper 10-cm depth in solar-heated plots (Table 1), the highest reduction of cabbage yellows occurred in that layer (Fig. 6). The effect may be the result of changes in soil microflora (3,4,10,13), diminished soil fungistasis (3), or release of nutrients that may induce chlamydospore germination leading to biological control of F. o. f. sp. conglutinans.

A number of cases have been reported where control of pathogens was obtained by solar heating, even though soil temperatures obtained by the treatment were insufficient to completely account for the effects obtained (4,10,12,13). Katan et al (4) suggested that suppressiveness in the solarized soils resulted from a shift in microbial populations in favor of heat-resistant antagonists. Also, the sensitive germinating propagules may be exposed to the action of antagonistic microorganisms and to other detrimental factors existing in the soil, as was shown with Armillaria mellea exposed to sublethal chemical (1) or thermal (9) treatments.

After treatment with solar heating plus cabbage amendments in the field, cabbage yellows was undetected, but when cabbage plants were planted in soil samples in the greenhouse from this plot, a significant amount of cabbage yellows developed on plants grown in soil from depths of 10-20 and 20-30 cm (Fig. 6). This was not surprising because assay of these soil samples (Table 2) revealed that the pathogen was present. The most likely explanation for failure of infection in field plots is that physical factors such as lack of aeration in the soil or fungistasis in the soil were limiting for germination and growth of the propagules of the pathogen. It may be necessary to free the soil of F. o. f. sp. conglutinans only in the upper 10- or 15-cm layer for successful field control of the disease. One question, however, needs to be resolved: Do

cabbage amendments after solar heating allow a shift in microbial populations in favor of antagonists? If they do, the effect of solar heating might be expected to last for several crop seasons.

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