Evaluation of Clear Polyethylene Mulch for Controlling Verticillium Wilt in Established Pistachio Nut Groves

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ABSTRACT

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Complete mulching of the soil surface of a 6-yr-old, drip-irrigated planting of pistachio nut trees with clear polyethylene sheeting for 2 mo resulted in elimination of *Verticillium dahliae* (undetectable amounts to 0.07 microsclerotia [MS] per gram in air-dry soil) to a depth of 120 cm. Initial inoculum densities at 30, 60, 90, and 120 cm were, respectively, 4.6, 0.9, 0.6, and 0.4 MS/g soil. Mulching was effective whether or not the soil was preirrigated with sprinklers before mulches were applied early in July. Complete mulching was much more effective than treatments in which polyethylene sheets were separated by 0.5 or 1.2 m at tree rows. One year following mulching in 1979, average infection percentages were 6.3, 1.6, 3.4, and 3.4, respectively, for the unmulched, completely mulched, and mulch treatments in which the polyethylene sheets were separated by 0.5 and 1.2 m in the tree rows. Thus, disease control was, at best, about 75%. Soil temperature at the 0–30 cm depth under mulches accounted for the demise of *V. dahliae*. Temperature did not, however, account for loss of MS at 60, 90 and 120 cm.

Additional key words: soil solarization, control of soilborne disease.

Control of pests with heating induced by mulching soils with polyethylene sheeting during hot weather was first done by Katan et al (7), although polyethylene mulches to warm soil during cool weather were used earlier (6). The literature on this procedure, now called soil solarization, was recently reviewed by Katan (6).

Mulching of existing plantings of trees is a departure from the standard soil solarization procedure, which has been used solely as a preplant treatment for annual row crops (6). We had four major preliminary reservations about success of the procedure; they were: the possibility of significant root damage due to elevated temperatures; the potential effect of shading, especially on the north side of trees; the potential impact of trees infected before mulching upon disease incidence following mulching; and the possibility of relatively deep-placed inoculum escaping the influence of solarization, since assay results (*unpublished*) showed that inoculum occurred as deep as 120 cm.

We report here on the use of polyethylene mulches to control *Verticillium dahliae* Kleb. in a pistachio planting in which trees ranged from 1–6 yr old. This planting, like a number of others, was made before the grower understood the threat of Verticillium wilt (3). In this case, approximately 85% of the original trees had been replaced within 6 yr. A need exists for a means of reclaiming such groves.

MATERIALS AND METHODS

Laboratory tests were made to determine the influence of temperature upon survival of *V. dahliae* microsclerotia (MS) in a naturally infested soil having an inoculum density (ID) of 14 MS per gram of air-dried soil. Three air-dry portions and three moist portions of the soil were incubated at 35, 40, and 45 C. Three 15-g samples of soil for both air-dried and moist soil and each temperature were assayed (2) periodically for 16 wk.

A first mulching experiment was begun 7 July 1978. It was patterned after those described by Katan et al (8). Water retention values for soil used in all experiments reported here were 33.3 and 18.5% for (respectively) the 1/3-bar and 15-bar water potentials, as determined by the standard pressure-membrane method. Soil had been furrow irrigated to thoroughly wet the soil profile through a depth of 120 cm. Drip irrigation proceeded as usual in the unmulched treatment, but the drip irrigation emitters were not operated for 3 wk after onset of the experiment where mulch was applied. The soil of three rows of trees (80 m long) was covered with clear polyethylene sheeting (150 μm thick). The sheets overlapped at trees in the center of rows. Outer edges of mulches were held in place by burying them in a shallow trench made earlier. Polyethylene sheets were held together where they lapped by small piles of soil. Three adjacent unmulched rows served as experimental controls.

Soil temperatures were continuously monitored at depths of 20, 40, and 60 cm in both treatments, in the unshaded area between rows, and in the partially shaded area near the drip line of the north side of trees.

ID was determined (5) for 0–20 cm depths of all treatments after 2 wk and for 0–20, 20–40, 40–60, and 60–80 cm depths after 6 wk. Soil samples were made up of 10 cores (2.5-cm diameter) taken approximately 8 m apart for each treatment row and for each depth. Each major sample was assayed in duplicate. Therefore, each grand mean was based on results for six assays.

Soils were reassayed at the 0–20 cm depth in August, 1979, and in January, 1981. A tree vigor determination was made by measuring new growth of five shoots on each of 20 trees per treatment in December, 1979. Too few trees were involved in this experiment (13 per replication) to assess possible disease control.

A second experiment was begun on 3 July 1979 in which there were five replicates of each treatment. Each replicate had 86 trees and was about 400 m long. Complete mulching, using 100-μm-thick clear polyethylene sheeting, was compared with treatments in which polyethylene sheets were separated by 0.5 and 1.2 m in tree rows. We also determined whether a wet soil profile (which was used before) was necessary by comparing an irrigated series, (using sprinklers instead of the furrow irrigation used before) with a series having only residual moisture from winter rainfall. There, except near the drip-irrigation emitters, soil moisture was at or below PWP (18.5% by weight), to a depth of about 1.2 m when the experiment was begun. Mulches were removed after 2 mo. Irrigation, following onset of the experiment, was done as before.

Soil temperature was continuously monitored in unshaded areas between unmulched and mulched rows at 30, 60, 90, and 120 cm depths. Inoculum densities were determined before mulching at depth ranges of 0–30, 30–60, 60–90, and 90–120 cm. After mulching, 10 cores were taken in unshaded and partially shaded

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TABLE 1. The influence of moisture and temperature upon survival of *Verticillium dahliae* microsclerotia incubated in field soil under laboratory conditions

Exposure period	Survival of microsclerotia (%)[a]				
	Air-dry soil		Moist soil		
	40 C	45 C	35 C	40 C	45 C
4 hr	···	···	100	14	3
24 hr	···	···	100	7	TR
32 hr	···	···	100	3	TR
1 wk	5	TR[b]	100	ND	ND
4 wk	2	ND[c]	100	ND	ND
8 wk	4	ND	100	ND	ND
11 wk	TR	ND	TR	ND	ND
16 wk	TR	ND	ND	ND	ND

[a] Percent of initial ID (14 MS/g soil).
[b] Trace; less than 0.5% of initial ID (14 MS/g soil).
[c] Not detected.

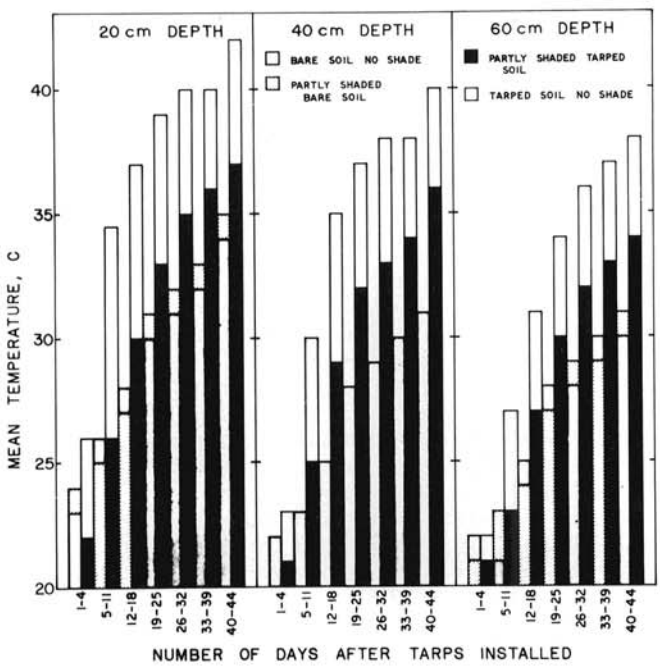

Fig. 1. The influence of clear, 150-μm-thick polyethylene mulches on temperatures of unshaded soil and soil (which was shaded part of each day) on the north side of a young pistachio tree in 1978.

areas of each row, as before, and at each of the above depths. The 10 cores for each depth in a row were taken about 40 m apart and were bulked into one major replicate sample. Assays were also made of the piles of soil used to anchor the mulches.

Observations on infection were made in mid-June 1980. As before, shoot growth measurements were made in December in the year following mulching. Five shoots on each of 20 trees per treatment were measured as before.

A third field experiment was begun on 1 December 1979 to determine whether mulching was effective at times other than the hottest months of the year. Inoculum densities were determined after 2 mo at 0–30 cm in five unmulched and five mulched rows approximately 70 m long; the latter covered with 100-μm-thick polyethylene sheeting as above. Irrigation was done as before. This test was repeated at monthly intervals from January through May, 1980. The last mulches were removed in early July.

To determine survival of MS, six samples of each unmulched and mulched soils were assayed according to the flotation procedure described by Huisman and Ashworth (5). Searches for MS in these preparations were made and viability of isolated MS was verified on pectate agar (4).

RESULTS

The influence of moisture and temperature upon survival of *V. dahliae* in field soils. Initially, the inoculum density was 14 MS/g soil; data are expressed as percentages of that population. The fungus was unaffected at 35 C in air-dry soil, but it was reduced to a trace (0.5% of the original amount) and was not detected after (respectively) 11 and 16 wk at 35 C in moist soil (Table 1). Eleven weeks at 40 C and 1 wk at 45 C were required to reduce the fungus to trace amounts in dry soil. In moist soil, however, 3% of the original inoculum of the fungus was detected after 32 hr at 40 C and only a trace of inoculum was detected after 24 hr at 45 C. *V. dahliae* was not detected at either temperature after 1 wk (Table 1.)

The influence of polyethylene mulches and shade upon soil temperature and growth of trees. Temperatures of unshaded and partially shaded soils were monitored in 1978. The amount of shading that occurred on the north side of young trees had little influence upon temperature of bare soil. There, differences between unshaded and shaded areas did not exceed 1–2 degrees (Fig. 1). Within a few days after mulching, however, differences of 5 C or more occurred between unshaded and partially shaded areas at depths of 20, 40, and 60 cm. These differences persisted throughout the 6-wk test (Fig. 1).

Soil temperatures of only unshaded soils were monitored at 30, 60, 90, and 120 cm depths in 1979 and 1980. Only 1980 data are reported here (Fig. 2A and B) because temperatures were monitored continuously from December 1979 to 27 August 1980

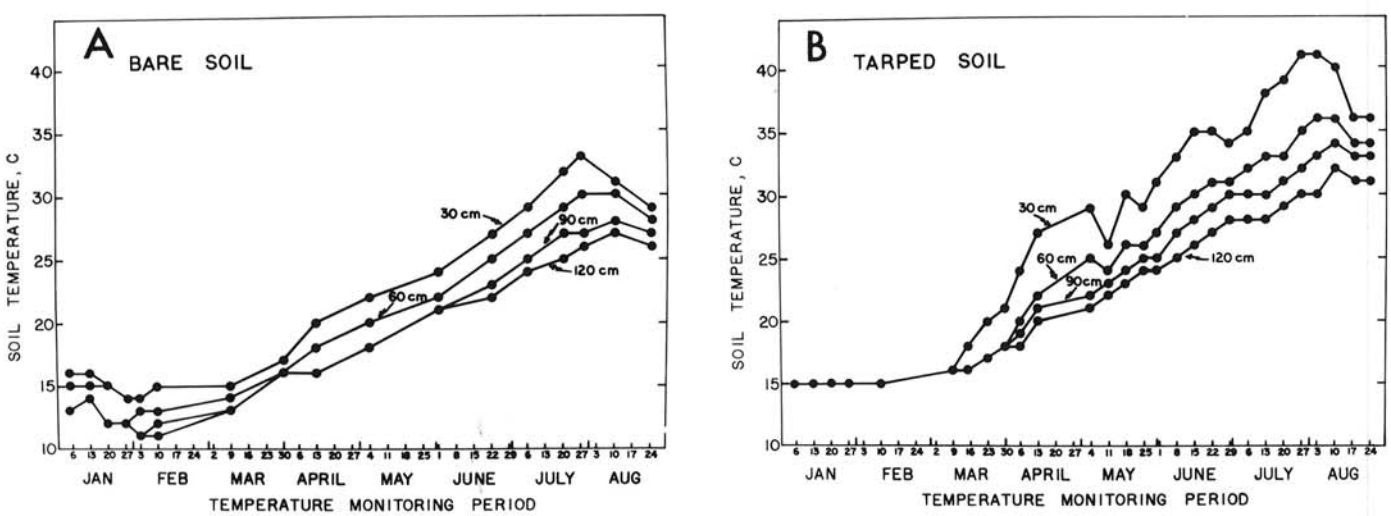

Fig. 2. Seasonal progression of temperatures of **A,** unshaded bare soil and of **B,** soil mulched with 100-μm-thick clear polyethylene in 1979.

opposed to 3 mo in 1979. Temperatures in both years were essentially the same in the July through August period.

Temperatures of soil at all depths (30, 60, 90, and 120 cm) rose steadily following 1 March and they were maximal, 42 C at 30 cm depth, during late July and early August (Fig. 2A and B).

Growth of trees was not measurably affected by mulching. One year following mulching in 1978, average shoot growth was 36–38 cm depending on treatment. It was 33–35 cm in 1980 following mulching in 1979.

The fate of *V. dahliae* and of infection of pistachio trees in bare and mulched soils. The ID of *V. dahliae* was unaffected after 2 wk at the 0–20 cm depths in 1978 except in the unshaded mulched treatment. There, only trace amounts of the fungus were detected. After 6 wk, the ID of *V. dahliae* was still unchanged in bare soil. On the other hand, only undetectable to trace levels of *V. dahliae* inoculum were detected in mulched soils to a depth of 80 cm, regardless of shading (Table 2). ID of the mulched rows in August 1979 and in January 1981 remained at trace levels (<0.05 MS/g soil), based on assays made on soil collected at the 0–30 cm depth.

ID measurements were made to a depth of 120 cm in 1979 instead of the 80-cm depth that was sampled in 1978. Complete mulching again was an effective control measure for *V. dahliae*. The fungus population density was severely reduced to a depth of 120 cm. This was true whether soil was moist to that depth from preirrigation or whether soil was, except near drip-irrigation emitters, at or below PWP (Table 3). The treatment in which polyethylene sheets were separated by 0.5 m appeared to be more effective in the space between polyethylene sheets at the 30-cm depth than where the polyethylene sheets were separated by 1.2 m. But, significant numbers of MS of the fungus persisted in soil at all depths in the spaces between polyethylene sheets. In soil under mulches, however, decrease in ID approached that observed in the completely mulched treatments (Table 3).

Twenty collections were made of the small piles of soil used to anchor the polyethylene sheets in place. An initial ID of 4.6 MS/g soil as observed in the 0–30 cm control was assumed (Table 3). The ID of individual samples ranged from undetectable to 1.2 MS/g soil but the mean was 0.3 MS/g soil.

We observed about 75% control of wilt disease in 1980 following complete mulching in 1979; eg, there was 6.3% infected trees (trees with external symptoms of wilt) in unmulched areas compared with 1.5–1.7% infection of trees in the completely mulched areas (Table 4).

We found that winter (December) and early spring (April) mulching was ineffective for control of *V. dahliae*. ID of the fungus was unchanged 2 mo after mulches were applied between December and April. Mulching in May was moderately effective in unshaded, but not in partly shaded areas. Mulching in June was not tested.

Occurrence of microsclerotia in unmulched and mulched soils. Qualitative observations on the survival of MS in mulched soils were made on soil collected 30–60 cm deep in fully mulched areas, 2 mo or more after the mulches were applied. Control soil had ~1 MS/g, Table 3; thus, about 15 MS were expected in each sieved (2) and centrifuged (5) preparation. From 2–12 MS were isolated from samples of control soil and their viability was verified by growing them on pectate agar (4), where they usually produced contaminant-free colonies, as reported earlier (1). No MS were recovered from six samples taken from mulched soils.

TABLE 2. The influence of clear polyethylene mulches on a reduction of inoculum (microsclerotia [MS]) density of *Verticillium dahliae* in soil after 6 wk during the summer of 1978

	Inoculum density (MS/g)[a]			
	Bare soil		Mulched soil	
Sampling depth (cm)	No shade	Partly shaded	No shade	Partly shaded
0–20	4.6	4.6	ND[b]	TR[c]
20–40	1.5	0.8	ND	TR
40–60	0.3	0.3	TR	TR
60–80	0.2	0.2	TR	TR

[a] Number of MS per gram of air-dry soil.
[b] Not detected.
[c] Trace; less than 0.05 MS per gram of air-dry soil.

DISCUSSION

Nelson and Wilhelm (10) showed that MS of *V. dahliae* in dry infested flower stalks of *Plantago lanceolata* were heat tolerant, but all were killed within 6 mo at 43–49 C and within 8 mo at 40 C. We observed that MS survived in trace amounts in naturally infested soil for at least 16 wk at 40 C while all MS appeared to be killed between 1–4 wk at 45 C. The temperature at the surface of mulches was not determined, but Katan et al (8) and Pullman et al (12) reported temperatures of 59–60 C at a depth of 5 cm beneath mulches. High temperatures, therefore, appear to account for the reduction of the ID of *V. dahliae* in the air-dry soil used to anchor mulches in these tests; eg, to 0.3 MS/g soil from 4.6 MS/g soil.

Temperatures under mulches appear to have been high enough for long enough in the 1978 experiment (Table 1) to account for reduction of ID of *V. dahliae* 40 cm deep in unshaded soil, but not in soil shaded part of each day (Table 2), where maximum temperatures of 36–37 C persisted for 4–10 days (Fig. 1). This was not long enough at 37 C, according to Pullman (11), to eradicate *V. dahliae* from naturally infested soil. Temperatures at 60 cm under mulches were not high enough or of sufficient duration, to account for the ID reductions observed (Table 2), whether mulches were unshaded or shaded part of each day (Fig. 1).

ID determinations in 1979 were made at 0–30, 30–60, 60–90, and 90–120 cm depths instead of 0–20, 20–40, 40–60, and 60–80 cm

TABLE 3. The influence of clear polyethylene mulches and pre-irrigation on reduction of inoculum, (microsclerotia [MS]) density of *Verticillium dahliae* after 2 mo during the summer in 1979

		Inoculum density, no. of microsclerotia/g of soil						
			Solid mulching		0.5 m Space between mulches		1.2 m Space between mulches	
Preliminary irrigation	Sampling depth (cm)	Unmulched control	Partly shaded	No shade	Partly shaded	No shade	Partly shaded	No shade
Yes	30	4.6	0.07	ND[b]	0.3	ND	3.7	TR
	60	0.9	TR[a]	ND	0.8	TR	0.9	TR
	90	0.6	TR	ND	0.8	TR	0.5	TR
	120	0.4	TR	ND	0.7	TR	1.1	TR
No	30	···	0.05	ND	0.1	ND	1.5	ND
	60	···	TR	ND	0.2	TR	0.8	0.1
	90	···	TR	TR	0.1	TR	0.5	0.1
	120	···	TR	ND	0.1	TR	0.7	0.1

[a] Trace; less than 0.05 MS per gram of soil
[b] Not detected.

TABLE 4. The influence of clear polyethylene mulches applied in summer, 1979, on control of Verticillium wilt of pistachio trees in 1980

Preliminary irrigation	Amount of infection by *Verticillium dahlieae* (%)			
		Space between mulches		
	Control	None	0.5 m	1.2 m
No	6.3	1.7	3.6	3.1
Yes	···	1.5	3.1	3.7

depths as in 1978. Soil heating 30 cm below unshaded mulches accounted for kill of *V. dahliae* (40–41 C for 2–3 wk [Fig. 2B and Table 2]). Based upon laboratory tests (Table 1), however, temperatures at 60 or more cm deep never were high enough to be lethal to MS of *V. dahliae* (Fig. 2B). Thus, the combined data of 1978 and 1979 indicate that temperatures at 40 cm, but not 60 cm, below unshaded mulches accounted for the changes in ID of *V. dahliae* observed (Table 1, Fig. 1 and 2B). Furthermore, temperatures 20 cm under mulches that were shaded part of each day do not account for changes in the ID of *V. dahliae* observed (Fig. 1 and Table 1).

Preliminary data suggest that MS under mulches are induced to germinate, largely suicidally, by a substance or substances that accumulate under mulches. Many substances that occur naturally in soil are known to relieve fungistasis (1,9). Thus, a low level of respiration of small roots due to the low temperatures during December–April mulching tests may account for lack of activity of mulches during that period. Conversely, respiration of small roots may account for the activity of the mulches applied May–July and later. A hypothesis of induced suicidal germination of MS is consistent with reductions of ID of *V. dahliae* observed in these experiments. Our data, however, do not preclude the possibility that MS were parasitized at depths greater than ~40 cm as a result of environmental conditions caused by mulching, although results of earlier tests showed MS to be essentially free from other microorganisms (1). Regardless, results of reassays ~12 and 18 mo after mulching show the treatment has potential for long-term control of *V. dahliae* in pistachio groves.

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