Solar Heating by Polyethylene Mulching for the Control of Diseases Caused by Soil-Borne Pathogens

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ABSTRACT

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Irrigated soils in the hot Bet-Shean and Jordan Valley regions were mulched with transparent 0.03-mm polyethylene sheets during the months of July or August, and soil temperatures were thereby increased. Different types of inocula of *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *lycopersici* were buried in mulched and nonmulched soils at various depths, recovered after certain time intervals, and their populations were estimated. After two weeks under polyethylene sheets, *V. dahliae* was eliminated at depths of 0 to 25 cm. The population of *F. oxysporum* f. sp. *lycopersici* at the depth of 5 cm was reduced by 94 to 100%; at 15 cm, 68 to 100%; and at 25 cm, 54 to 63%. Maximal temperatures in the mulched soils were 49 to 52 C and 42 C at depths of 5 and 15 cm, respectively. At 50 C, soil fungistasis to *Fusarium* was partially nullified. Two field experiments with eggplant and one with tomato showed, that mulching with polyethylene sheets prior to planting reduced Verticillium wilt by 25 to 95%, controlled weeds, improved plant growth and stand, and increased yield. This method of control using plastic material is less costly than fumigation, is nonhazardous, and leaves no residues. It is suggested that biological as well as thermal control may take place during soil mulching.

Additional key words: heat treatment, *Lycopersicon esculentum, Solanum melongena,* weeds, wilt.

The search for new, inexpensive and nonhazardous methods for controlling diseases caused by soil-borne pathogens is a continuous task, as none of the many existing methods can be used in all instances. Fumigation, though effective and commonly used in Israel in some crops, is expensive, and hence economically restricted to certain crops and seasons. Heat treatment of soil by steam (or other means) for the control of such diseases has never been widely used, owing to economic considerations.

The use of plastic materials to protect crops in tunnels or greenhouses or for soil mulching is very common in Israel. Local growers and instructors of the Extension Service in the hot Bet-Shean and Jordan Valley regions have noted that mulching soil with polyethylene sheets in the hot seasons increased soil temperatures, due to solar heating.

In this study we describe the use of polyethylene sheet mulching in irrigated soils for the control of diseases caused by soil-borne pathogens by natural heating. A brief report of some of these results has been published (11).

MATERIALS AND METHODS

Mulching.—Mulching was used on soils moistened by irrigation for the purpose of increasing thermal sensitivity of resting structures (5, 6, 13) and improving heat conduction (4). A drip system was laid on tilled soil before mulching plots with transparent polyethylene plastic sheets (0.03 mm). Immediately after mulching, both mulched and nonmulched plots were irrigated and were maintained wet by repeated irrigations at 3- to 6-day intervals until mulching was removed. The drip method enabled us to irrigate the soil without removing the polyethylene sheets. Soil temperature was measured and recorded using mercury-in-steel distance type thermographs (Negretti and Zambra, U.K.).

Inoculum.—The following inocula were incorporated into the soil to various depths and for the indicated periods:

Fusarium oxysporum f. sp. *lycopersici* (Sacc.) Snyd. & Hans., race 1 (ATCC 28071) or race 2.—1) Soil.—Natural loamy sand soil was infested with conidia of the pathogen and kept wet for 12 weeks. Direct microscopic examinations revealed that by the end of this period only chlamydospores remained in the soil.

2) Plant material.—Diseased tomato seedlings obtained by artificial inoculation (1) were used either as dried ground stems sieved to 0.97 mm, or as roots or stems 2-4 cm long (root and stem pieces after removal from the soil were cut into 5-mm-long segments for plating on the assay medium).

Verticillium dahliae Kleb.—1) Wheat straw.—Autoclaved pieces 5-mm long were infested with conidia of the pathogen and incubated at 24 C. After 3 weeks they were densely colonized with microsclerotia and conidia. The conidia were removed by washing the straw pieces with running water. The straw pieces were then dried, ground and sieved to 0.97 mm.

2) Tomato.—Dried stems of diseased tomatoes from naturally infested soils were collected from the field at the end of the season, when heavily covered with microsclerotia of the pathogen, and were ground to 0.97 mm.

Populations of *F. oxysporum* f. sp. *lycopersici* were either directly assessed using the dilution method, or estimated as percentage of colonized plant pieces, both on PCNB-peptone medium (1). *Verticillium dahliae* populations were estimated in a similar manner, by incubation at 18 C on a selective medium, PCNB-ethanol (2).

Percentage of pathogen control due to mulching was calculated as

$$(1 - \frac{B}{A}) \times 100$$

where A = number of propagules per gram or percent colonization of plant pieces in the nonmulched soil; and B = the corresponding data in the mulched soil.

Fungistasis.—Effect of soil heating on fungistasis was tested with *F. oxysporum* f. sp. *lycopersici*. Natural loamy sand soil at 50% moisture holding capacity (MHC) was heated to the desired temperature in a controlled-temperature water bath. Drops of a suspension of twice-washed conidia of the pathogen (3×10^6/ml) were put on 0.45-μm membrane filters, 13 mm in diameter (Sartorius, Germany), and covered with another filter. The membranes were buried to a depth of 5 mm in the test soil which had been cooled and placed in petri dishes. After 24 hours of incubation, membranes were recovered and adhering soil was removed. Membranes were then stained with aniline blue in lactic acid and the percentage of germination was determined. A total of 400 conidia per treatment was counted.

Field experiments.—Three field experiments to study the effect of soil mulching on control of Verticillium wilt were carried out in naturally infested soils, in the Jordan Valley with eggplants [(*Solanum melongena* L. 'Black Beauty'), experiments 1 and 2], and in the Bet-Shean Valley with tomatoes [(*Lycopersicon esculentum* Mill. 'Rehovot 13'), experiment 3]. Soil types were either desert alluvial (experiments 1 and 2) or rendzina soil of valley (experiment 3). Experiments 1 and 2 consisted of a randomized block design and had six and five replicates, respectively, consisting of three rows 12 m long each, 1.5 m margins included. Experiment 3 consisted of two replicates, each with three rows 15 m long. Spaces between plants were 50 × 150 cm in experiments 1 and 3 and 40 × 140 cm in experiment 2. Special care was taken to keep soil in good tilth, allowing close contact between the plastic sheets and the soil and preventing the formation of 'air pockets' which reduce heat conduction. Soil was mulched either by machine (experiment 1) or manually. Mulching was carried out in strips 70 cm wide; hence, only 40 to 50% of the soil was treated. Dates of mulching were 22 August 1974 (experiments 1 and 2) and 5 September 1974 (experiment 3). Eggplant and tomato seedlings 30 days old were obtained from a commercial nursery, where they had been grown in a pathogen-free soil. The polyethylene sheets were removed and the seedlings transplanted in rows, in the middle of the polyethylene strip covered areas. Dates of planting were 30 September 1974 (experiments 1 and 2) and 2 October 1974 (experiment 3).

Verticillium wilt severity was estimated several times during the season on a 0-to-4 scale, where 0 = healthy plants and 4 = plants completely wilted and desiccated. Results were expressed both as percent of diseased plants and as an average disease index. Isolates made from randomly selected diseased plants confirmed the presence of *V. dahliae*.

TABLE 1. Effect of 14 days of soil mulching with polyethylene sheets on populations of *Fusarium oxysporum* f. sp. *lycopersici* (race 1 or 2) (F.o.l.) or *Verticillium dahliae* (V.d.)[a]

				Population reduction (%) at depths of:		
Pathogen	Race	Inoculum[b]	Test method[c]	5 (cm)	15 (cm)	25 (cm)
F.o.l.	1	Soil	Dilution	100	100	...
	1	Soil	Dilution	95	68	63
	2	Ground stems	Dilution	100	100	...
	2	Root segments	Plating	97	100	74
	2	Stem segments	Plating	94	67	54
V.d.	...	Wheat straw	Plating	97	97	...
	...	Tomato	Plating	100	100	...

[a]Results are expressed as percent reduction of the pathogen in the mulching treatment by calculating (1 − B/A) × 100 where A = number of propagules per gram or % colonization of inoculum units by the pathogen in the nonmulched soil; and B = the corresponding data in the mulched soil.

[b]Various types of inocula of the pathogen were incorporated either in mulched or in nonmulched soils at various depths. Inocula were: (i) Soil = soil containing chlamydospores of the pathogen. (ii) Ground stem = stems of diseased tomato seedlings dried and ground. (iii) Root, stem = segments of roots or stems of diseased tomato seedlings. (iv) Wheat straw = sterile inoculated segments containing microsclerotia. (v) Tomato = ground stem tissues of diseased tomato plants containing microsclerotia of the pathogen.

[c]Dilution = dilution plate technique. Plating = plant segments plated on the respective selective media and percent colonization determined.

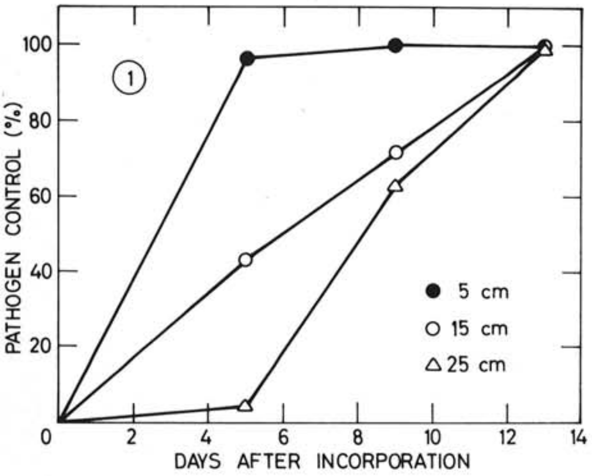

Fig. 1. Effect of soil mulching with transparent polyethylene sheets on the control of *Verticillium dahliae*. Microsclerotia of the pathogen (on wheat straw inoculum) were incorporated at various depths either in mulched or in nonmulched soil. Samples were removed after various periods and percent colonization by the pathogen was estimated. Results are expressed as percent control of the pathogen in the mulching treatment by calculating $(1 - B/A) \times 100$, where A = % colonization of inoculum units by the pathogen in the nonmulched soil, B = the corresponding data in the mulched soil.

RESULTS

Effect of soil mulching or heating on the viability of inocula of Fusarium oxysporum f. sp. lycopersici and Verticillium dahliae.—Inocula of the two pathogens were incorporated to various depths in the soil in the Jordan Valley during the month of July, 1973. Their viability in the mulched soil was compared to that in the nonmulched soil. Results (Table 1) show a pronounced reduction of pathogens by mulching: 94 to 100% reduction at the upper layer and 54 to 100% reduction at the lower layers. In another experiment (Fig. 1) we showed that extending the exposure of *V. dahliae* inoculum in the mulched soil further reduced the percentage of the pathogen in the soil; after 13 days a 100% reduction of the inoculum was achieved at all depths. The results in Table 1 and Fig. 1 may be correlated with temperature measurements (Table 2, 3). As expected, the highest soil temperatures (up to 52 C) were in the upper layer. The greatest temperature differences between mulched and nonmulched soils were at the peak temperatures. Soil temperature measurements also revealed a duration of maximal temperature of two or more hours daily. Temperatures below 35 C, still in the range of microbial activity, lasted 12 hours or more each day under mulching. Air temperature measurements (Table 3) showed that although maximal temperatures are quite similar during July to September, periods of unusually high temperatures are more likely to occur in July.

Soil moisture greatly affected sensitivity of the resting structures to heat treatment. In various experiments carried out in a controlled-temperature water bath, moistened soil-inoculum of *F. oxysporum* f. sp. *lycopersici* or moistened sclerotia-inoculum (from tomato) of *V. dahliae* were eradicated by 95 to 100% and 100%, respectively, after an incubation period of 1 hour at 50 C. In air-dried soil, viability of *V. dahliae* was reduced only partially by 50 C for 6 hours or at 55 C for 1 hour. Viability of *Fusarium* was not affected by 55 C for 1 hour in air-dried soil.

Experiments were carried out to examine the possibility that heating to 45 to 50 C affects fungistasis of the pathogen and microbial activities which may influence pathogen populations. Conidia of *F.*

TABLE 2. Soil temperatures in the Jordan Valley in 1973

			Soil temperature (C)					
			Polyethylene-mulched soil			Nonmulched soil		
Year	Month	Depth (cm)	Maximal (absolute)	Maximal (avg)	Minimal (avg)	Maximal (absolute)	Maximal (avg)	Minimal (avg)
1973	July	5.0	52	50.7	25	42	37.6	25
		15.0	42	40.8	33	34	32.4	31
	Aug 22	5.0	49	46.3	31	41	38.7	21
	up to	12.5	42	39.8	...	37	34.1	...
	Sept 15	20.0	38	37.0	35	33	30.8	27

TABLE 3. Air temperatures in the Jordan Valley of Israel in 1973 and 1974

		Air temperature (C)[a]			
Year	Month	Maximal (avg)	Minimal (avg)	Monthly (avg)	No. of days > 40 C (max)
1973	July	39.2	23.5	31.2	12
	August	37.9	24.6	30.7	4
	September	37.9	23.5	30.5	5
1974	July	37.7	23.8	30.8	6
	August	36.6	27.0	29.3	3
	September	37.6	22.5	30.1	3

[a]Data kindly supplied by the Israel Meteorological Service.

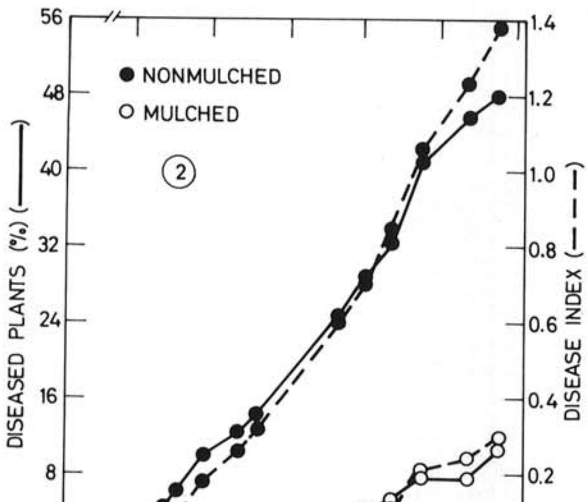

Fig. 2-3. Effect of soil mulching with transparent polyethylene sheets on percent diseased plants and disease index (0 to 4 scale; 0 = healthy plants) by *Verticillium dahliae* in eggplants. Differences between mulched and nonmulched treatment at each testing period and with both criteria are significantly different ($P = 0.05$). Field experiments 1 (**Fig. 2**) and 2 (**Fig. 3**) were carried out in the Jordan Valley.

oxysporum f. sp. *lycopersici* were incubated in a preheated natural loamy sand soil and percentage of germination was determined. Germination in soil that was not pretreated was 19%, whereas germination in soils that were preheated at 45 C for 3 hours, at 50 C for 1 hour and at 50 C for 3 hours was 38%, 50%, and 63%, respectively. Germination in autoclaved soil (1 hour at 1 atm) was nearly 100%. These experiments were repeated twice with similar results.

Natural loamy sand soil samples at 50% were treated for 3 hours at room temperature, at 45 C and at 50 C. After soils were cooled, conidia of *F. oxysporum* f. sp. *lycopersici* were mixed at a final rate of 4,000/g and population counts at various periods were determined using the dilution technique. Percent reduction in populations (as compared to zero time) after 3 and 9 days, were 0 and 24 in the nonheated, 20 and 51 for the 45 C treated, and 50 and 81 for the 50 C treated soils, respectively.

Effect of soil mulching on the control of Verticillium wilt in field experiments.—Three field experiments were carried out in the hot regions of Bet-Shean and the Jordan Valley in 1974 in naturally infested soils. Although July is the optimal mulching time in Israel, mulching was carried out in August and September, owing to technical difficulties. Mulching for 4-5 weeks resulted in a pronounced and highly significant reduction in both incidence of disease and disease index, throughout the growing season (Fig. 2-4). Best control was achieved in experiment 1, which was carried out in naturally lightly infested soil. In this experiment, disease incidence was significantly reduced by 80 to 95% during the various periods of plant growth.

Almost complete control of weeds, e.g. *Alhagi maurorum* Medik., *Cyperus rotundus* L., *Notobasis syriaca* (L.) Cass., and *Prosopis farcata* (Banks et Sol.) Eig, was observed in the three experiments. In addition, both growth and stand of the plants were higher in the mulched plots. For example, in experiment 2, plant height increased by 38% and stand increased by 26%, 58 days after planting (significant differences, $P = 0.05$). Yield and average weight of eggplant fruit in the mulched plots in experiment 2 were 11,475 kg/ha and 203 g respectively, while the corresponding numbers in the nonmulched plots were 3,640 kg/ha and 162 g (significant differences between treatments, $P = 0.05$).

DISCUSSION

Mulching with polyethylene sheets increased soil temperature and resulted in pronounced reduction in the populations of *V. dahliae* and *F. oxysporum* f. sp. *lycopersici* as well as in disease caused by *V. dahliae* in eggplants and tomatoes. This method resembles soil fumigation or heat treatment in that it has additional beneficial side effects; e.g. weed control and improvement of the growth and stand of the plant. However, soil mulching is superior to fumigation, being much cheaper, safer, involving no phytotoxicity or pesticide residues, and not requiring sophisticated machines. Mulching can be carried out either mechanically on a large scale, or manually. Its low cost enables extending its use to a large spectrum of crops. Besides, being cheaper, this treatment reduces hazards of phytotoxicity occurring at high temperatures (9) and is less detrimental to the biological equilibrium in the soil. It should be noted here that heat treatment of soils for the control of diseases caused by soil-borne pathogens, e.g. by aerated steam, is now being carried out at temperatures lower than those used in the past (6).

Temperatures achieved at the upper soil layers by mulching are in the range of those found to be lethal to the pathogens (13). At lower soil layers, where the temperatures were lower, the pathogen populations were also reduced. This may be due either to a direct cumulative effect of sublethal temperature on the pathogens, or to a combination of thermal and biological

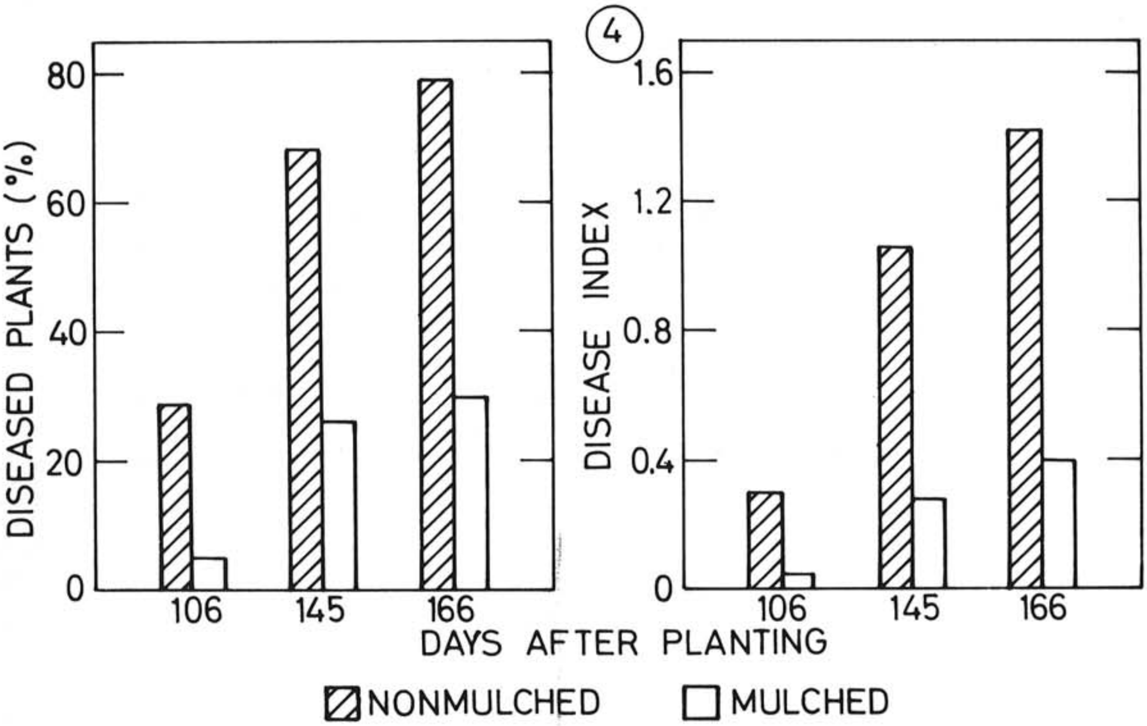

Fig. 4. Effect of soil mulching with transparent polyethylene sheets on percent diseased plants and disease severity (0 to 4 scale; 0 = healthy plants) by *Verticillium dahliae* in tomato plants. Field experiment 3 was carried out in the Bet-Shean Valley. In all cases, mulched treatments were significantly different from the nonmulched controls ($P = 0.05$).

control. There are at least three ways in which such a biological control can operate in the mulched soil. (i) Fungistasis, which keeps the fungus propagules at a passive resistant stage, is partially nullified at 45 to 50 C. Thus, the sensitive germinating propagules are exposed to the action of lytic microorganisms and to other detrimental factors existing in the soil. Our results, which show increased germination rate of *Fusarium* conidia in preheated soil, are in agreement with those of Dobbs and Hinson (10) who found increased germination of spores of *Penicillium frequentatus* in soil preheated at 50 C for 1 hour. Even though conidia of *Fusarium* are not the main resting structure in natural soil (12), their germination in previously heated soil indicates a release of nutrients (14). This also explains the greater decline of populations of *Fusarium* in previously heated soil. (ii) Sublethal temperature may weaken the resting structures, rendering them more vulnerable to the antagonistic microflora as was shown in *Armillaria mellea* exposed to sublethal chemical and thermal treatments (7). (iii) Creation of a shift in microbial populations in the soil in favor of heat resistant saprophytes. This is expected as most pathogens are generally less resistant to heat than many saprophytes (5, 6, 7, 8). One beneficial consequence of the microbial population shift in mulched soil would be its protection from reinfestation. Although the strips of mulched soils comprised only 40 to 50% of the soil area, and were surrounded by infested soil, control of *V. dahliae* disease lasted to the end of the season (166 days in experiment 3). It is assumed, that the newly established microflora prevented reinfestation, although further investigation is still needed to establish this theory.

Our results were obtained in the hot regions of the Bet-Shean and Jordan Valleys. However, the implications extend beyond this specific case. Mulching for disease control might be effective in regions with lower temperatures if its effectiveness is increased, e.g. by mulching the whole field instead of by strips, by extending the mulching period, or by using other types of plastic sheets. It will be interesting, therefore, to examine this method in other regions, with other crops and diseases, or in combination with fumigation or some other chemical treatment used at a low dosage. In all cases, pathogen-free seedlings should be used. It is expected that higher temperatures will be reached by mulching in regions with higher solar radiation intensity. In glasshouses, this method (with additional heating, if necessary) might be especially easy to handle.

Although the use of polyethylene for mulching in agriculture is a relatively new technique, the idea of using solar heating for plant protection is by no means new in this country. Mulching of tomato seedbeds with sawdust was used in the Jordan Valley to increase air temperature above the sawdust to over 46 C which prevented infestation of tomato seedlings with the tobacco white fly (*Bemisia tabaci*), the vector of the tomato yellow leaf curl virus (3).

LITERATURE CITED

1. ALON, H., J. KATAN, and N. KEDAR. 1974. Factors affecting penetrance of resistance to Fusarium

oxysporum f. sp. lycopersici in tomatoes. Phytopathology 64:455-461.
2. AUSHER, R., J. KATAN, and S. OVADIA. 1975. An improved selective medium for the isolation of Verticillium dahliae. Phytoparasitica 3: (In press).
3. AVIDOV, Z. 1956. Bionomics of the tobacco white fly (Bemisia tabaci Gennad.) in Israel. Ktavim 7:25-41.
4. BAKER, K. F. (ed.) 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23:1-332.
5. BAKER, K. F. 1962. Principles of heat treatment of soil and planting material. J. Aust. Inst. Agric. Sci. 28:118-126.
6. BAKER, K. F. 1970. Selective killing of soil microorganisms by aerated steam. Pages 234-239 *in* T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. Root diseases and soil-borne pathogens. University of California Press, Berkeley and Los Angeles. 252 p.
7. BAKER, K. F., and R. J. COOK. 1974. Biological control of plant pathogens. W. H. Freeman, San Francisco. 433 p.
8. BROADBENT, P., K. F. BAKER, and Y. WATERWORTH. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. Aust. J. Biol. Sci. 24:925-944.
9. DAWSON, J. R., R. A. H. JOHNSON, P. ADAMS, and F. T. LAST. 1965. Influence of steam/air mixtures, when used for heating soil, or biological and chemical properties that affect seedling growth. Ann. Appl. Biol. 56:243-251.
10. DOBBS, C. G., and W. H. HINSON. 1953. A widespread fungistasis in soils. Nature 172:197-199.
11. KATAN, J., A. GREENBERGER, H. ALON, and A. GRINSTEIN. 1975. Increasing soil temp by mulching, for the control of soil-borne diseases. Phytoparasitica 3:69-70. (Abstr.).
12. NASH, S. M., T. CHRISTOU, and W. C. SNYDER. 1961. Existence of Fusarium solani f. phaseoli as chlamydospores in soil. Phytopathology 51:164-166.
13. NELSON, P. E., and S. WILHELM. 1958. Thermal death range of Verticillium albo-atrum. Phytopathology 48:613-616.
14. STEINER, G. W., and J. L. LOCKWOOD. 1969. Soil fingistasis: Sensitivity of spores in relation to germination time and size. Phytopathology 59:1084-1092.