Physical, Biological, and Chemical Control Integrated for Soilborne Diseases in Potatoes

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ABSTRACT

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Solar heating of the soil (by polyethylene mulching) or the fumigant methyl bromide, when tested under field conditions, significantly reduced diseases caused by *Rhizoctonia solani* and *Verticillium dahliae*. The biocontrol agent *Trichoderma harzianum* reduced *R. solani* disease. Combining solar heating or methyl bromide with *T. harzianum* improved their efficiency and also resulted in the control of *Sclerotium rolfsii*. The methyl bromide treatment significantly increased the yield by 64% in one experiment. Both *T. harzianum* and solar heating reduced the inoculum potential of *R. solani* and its build-up in the field and under greenhouse conditions. This was more pronounced when the two treatments were combined. Vapam, methyl bromide or solar heating killed 76-100% of the *S. rolfsii* sclerotia buried in the soil during treatment. Combining heat treatment and *T. harzianum*, both at sublethal doses and under greenhouse conditions, resulted in 90-100% control of *S. rolfsii* disease in beans. Integrated control of soilborne diseases in potatoes was achieved by an optimal combination of physical, chemical, and biological means.

Additional key words: pentachloronitrobenzene, ammonia, soil solarization.

Soilborne diseases of potatoes may cause heavy losses to this crop at all stages of growth: seedling, maturing plants, and tubers. A significant reduction in crop loss can be obtained by maintaining inoculum density or potential low for several months, thus protecting the plant throughout its growth period. Fungicides, such as pentachloronitrobenzene (PCNB), may be effective in controlling *Rhizoctonia solani* and *Sclerotium rolfsii* both in potato shoots and tubers (1,3,11). Fumigants, eg, methyl bromide and vapam, are widely used for controlling a broad spectrum of soilborne pathogens in various crops (6,13,14,20), though in certain cases their cost may be a prohibitive factor. Ammonia might be effective in controlling *S. rolfsii* (1). *Trichoderma harzianum* was effectively used as a biocontrol agent against *R. solani* and *Sclerotium rolfsii* (2,5,19) under both greenhouse and field conditions.

In recent years a new approach for controlling soilborne diseases, by solar heating of the soil, was developed in Israel (4,8,9). This is carried out by mulching the moistened soil during the hot season with transparent polyethylene sheets, thereby increasing soil temperatures and controlling pathogens and weeds. In potatoes, *Verticillium dahliae* and *Pratylenchus thornei* were effectively controlled by this method (4). Various pathogens were controlled by this method in California (18). An integrated control, using an optimal combination of various means of disease control and minimizing pesticide dosage, may, as indicated with *R. solani* and *S. rolfsii* (2,5,7), improve disease control and reduce environmental hazards.

In the present work various methods (alone or combined) for the control of soilborne diseases of potatoes, were studied under field and controlled conditions. Changes in fungal populations and inoculum build-up were also followed.

MATERIALS AND METHODS

Field experiments. Two experiments with potatoes were carried out in 1977 and 1978 in the northern region of Israel in fields of an alluvial vertisol soil (27% sand, 17% silt, 55.5% clay, and 0.5% organic matter; pH 7.95; moisture holding capacity 40%) where potatoes were frequently grown. The 1977 experiment, carried out in five replicates, consisted of a randomized block design containing six rows, each 14 m long and 80 cm apart. Potatoes (*Solanum tuberosum* L. 'Up-to-date') were sown on 17 August 1977 and harvested on 28 December 1977. In the similarly designed 1978 experiment, potatoes were sown on 20 August 1978 and harvested on 21 December 1978. The 1977 experiment consisted of three subexperiments due to the large number of treatments.

Surface disinfested, certified tubers were used for sowing. Samples of diseased plants were plated out on potato dextrose agar (PDA), incubated at both 24 and 27 C and the growing fungi were identified.

Mulching was carried out from 5 July 1977 till 13 August 1977 in the first experiment, and from 26 June till 30 July 1978 in the second. Soil was kept moist during mulching in order to increase thermal sensitivity of resting structures and improve heat conduction. In the 1977 experiment a drip system was laid on the tilled soil and 3 days after the first irrigation the soil was mulched with transparent polyethylene sheets (0.05 mm thick). The drip (trickle) system consisted of perforated plastic tubing from which water delivered at a constant flow rate. Four additional irrigations were given during the mulching period by means of the drip system. In the 1978 experiment the drip system was not used, and the soil was kept moist by means of a single irrigation given before mulching (4). Maximal temperatures of the mulched soil were 45–55 C and 40–45 C at depths of 5 and 20 cm, respectively. Soil temperatures in the nonmulched plots were 7–14 C lower.

An isolate of *T. harzianum* Rifai aggr., capable of attacking both *R. solani* Kuehn and *S. rolfsii* Sacc., was used. It was grown for 14 days at 30 C on a wheat bran:sawdust:tap water mixture (3:1:4, w/w/v) which was autoclaved, in autoclavable plastic bags, for 1 hr at 121 C on 2 successive days. In the field experiment, a 40 cm-wide strip of this preparation was spread in each row on the day of sowing at the rate of 400 or 600 kg/ha, and then incorporated into the soil to a depth of 10 cm by means of a rotary hoe.

Terraclor (16 kg/ha, 75% pentachloronitrobenzene wettable

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powder, Olin Chemicals, Little Rock, AR 72200 USA) was sprayed on the soil of the whole plot and then incorporated as described with *T. harzianum.* Methabrom (98% methyl bromide, Bromine Compounds, Beer-Sheva, Israel, 350 or 500 kg/ha) treatment was carried out on 1 August 1977 in the first experiment and on 29 June 1978 in the second. Edigan (32% Metam-sodium (Vapam), Agan Chemicals, Ashdod, Israel, 300 L/ha) was applied to the soil, to a depth of 40 cm, by means of sprinkler irrigation on 4 August 1977. Ammonia solution (Fertilizers and Chemicals, Haifa, Israel, 2,000 L/ha) was injected into the soil of the whole plot on 28 August 1978.

Before planting, the soil was fertilized with 1,000 kg/ha superphosphate and 200 kg/ha ammonium sulphate. During the growth period, the plants were fertilized four times with ammonium phosphate (total application 750 kg/ha). The plants were irrigated and treated with insecticides (methomyl, diazinon, chlorpyriphos, and azinphos methyl) according to the commercial practice in this region. Stand counts, carried out 50 days after planting, were within range of 30–35 plants per 10 m, with no significant differences between treatments.

At the indicated periods, 10 plants from each replicate were lifted and examined for the presence of *R. solani* lesions on the hypocotyls and xylem discoloration in the stems caused by *V. dahliae.* The presence of these pathogens was verified by plating out plant tissues on potato dextrose agar. Results were expressed as percentage of diseased plants.

Population of *Trichoderma* propagules in the soil was estimated by the soil dilution method and a Martin's medium, containing rose bengal (12).

Greenhouse experiments. The inoculum potential of the general population of *R. solani* in soil was estimated using beans (*Phaseolus vulgaris* L. 'Brittle Wax') as a test plant. Bean seeds were sown in 10 × 10 × 6 cm plastic boxes containing soil samples from the field (nine seeds/box; seven replicates) and maintained in the greenhouse at 24–30 C for 28 days. Diseased plants were recorded and at the end of the experiment all remaining plants were uprooted and examined for symptoms of diseases caused by *R. solani.* Artificial infestation was carried out with sclerotia of *S. rolfsii* (0.1 g/kg soil unless otherwise stated) taken from 14-day-old cultures grown on a synthetic medium (17). Wheat bran inoculum preparation of *T. harzianum* was mixed with the soil at the rates of 2 or 4 g/kg soil.

Wet sandy soil (14% moisture) was mixed with *S. rolfsii* sclerotia and was heated at 25, 47, and 50 C for 2 hr on four successive days in Wisconsin temperature-controlled tanks and then sown with bean seeds and maintained in the greenhouse. Disease incidence was estimated during 21 days.

RESULTS

Control of soilborne diseases in potatoes—field experiment 1977. Plant samples were dug twice during the growing season and the percentage of diseased plants was determined. *R. solani* disease in shoots was evident in the control plots, and its incidence increased with time (Table 1). Solar heating (with or without the addition of *T. harzianum*), methyl bromide, and *T. harzianum* alone were the only treatments that significantly reduced disease incidence at 54 days after planting. At 86 days after planting, a significant reduction in the disease was achieved by solar heating, *T. harzianum,* or both. Disease incidence in tubers, determined at harvest, about 135 days after planting, was significantly reduced by the solar heating treatments only (Table 1).

Two months after planting, *V. dahliae* was isolated from wilted plants. Only the two solar heating treatments and the methyl bromide treatments significantly reduced the incidence of Verticillium wilt (Table 1). Although plants in plots that received these treatments showed a trend towards increased yield (range, 34–64%), the increase was statistically significant ($P = 0.05$) only with the methyl bromide treatment, apparently due to the large variation in the field. Weed population, as estimated by visual appearance at field, was much reduced by solar heating and methyl bromide.

Effect of soil treatments on *Trichoderma* spp. populations, inoculum potential, and disease buildup. Populations of *Trichoderma* spp. were assayed (at 2 and 135 days after planting) in soils from four treatments in the 1977 experiment. The natural population of this potential antagonist in the control soil was low; ie, 10 to 15 propagules per gram. Solar heating increased *Trichoderma* population up to 350 propagules per gram at 135 days after planting. As expected, *Trichoderma* populations were much higher after *T. harzianum* was added to the soil (alone or combined with a solar heating treatment) reaching 8.9×10^6 and 4.0×10^4 propagules per gram at 2 and 135 days after planting, respectively.

Soil samples from various plots of the 1977 experiment were brought to the greenhouse at 0, 90, and 135 days after planting. Bean seeds were sown in these samples and grown for 28 days. The percentages of *R. solani*-diseased plants were assessed in order to determine the inoculum potential of the general population of *R. solani* in the soil. After potatoes were planted, *R. solani* inoculum potential increased with time in the infested control plots, apparently due to the potato host influence. The inoculum potential at planting day was reduced by the other three treatments (Fig. 1). In addition, solar heating (especially when combined with *T. harzianum*) delayed inoculum buildup during the season. These results (Fig. 1) are in agreement with those regarding *R. solani*

TABLE 1. Effect of field soil treatments and *Trichoderma harzianum* on diseases and yield of potatoes[x]

Treatments	Dosage (kg/ha)	Post plant disease symptoms (%) caused by: *R. solani* in shoots (54 days)	*R. solani* in shoots (86 days)	*R. solani* in tubers (133 days)	Verticillium wilt (86 days)	Total yield (kg/ha)
Subexperiment 1						
Infested control	···	44 a[y]	72 a	62 a	44 a	6,637 a
Solar heating (SH)	···	12 b	23 b	26 b	9 b	8,889 a
T. harzianum (TH)	400	22 ab	36 b	47 ab	34 a	7,341 a
SH + TH	0 + 400	0 b	18 b	32 b	3 b	9,904 a
Terraclor	16	20 ab	48 a	45 ab	28 a	7,052 a
Subexperiment 2						
Infested control	···	37 a	75 a	60 a	20 a	7,963 a
Methyl bromide	350	12 b	36 a	52 a	4 b	13,055 b
Methyl bromide	500	3 b	45 a	62 a	6 b	11,528 b
Subexperiment 3						
Infested control	···	45 a	60 a	64 a	58 a	7,438 a
Metam-sodium	300[z]	35 a	20 a	72 a	31 a	8,735 a

[x] Field experiment, 1977, in northern Israel.
[y] Numbers in each column followed by a common letter are not signficantly different ($P = 0.05$).
[z] Dosage in liters per hectare.

disease control in the field by the same treatments (Table 1).

The capacity of *R. solani* inoculum to increase in soil samples from the field was tested in the greenhouse by using another approach. After determining *R. solani* disease incidence in bean seedlings grown in these soil samples, the soil from each treatment was mixed and bean seeds were sown again. This procedure was repeated twice (second and third planting). Successive sowing of beans in soil from the infested control plots at the first sampling, increased the inoculum potential (Fig. 2) suggesting a buildup of inoculum (10). The other three soil treatments slowed down the increase in inoculum potential of *R. solani*.

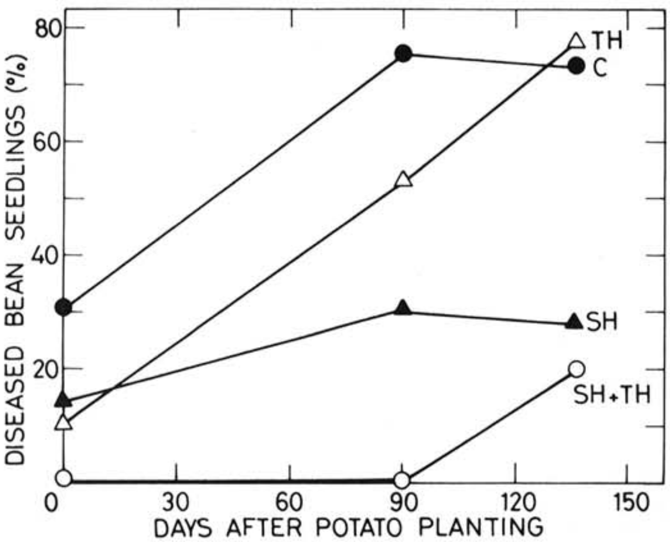

Fig. 1. Incidence of *Rhizoctonia solani* disease in bean seedlings sown in soil samples taken from plots in a field experiment at 0, 90, and 135 days after planting with potatoes. The treatments in the field were: Infested control (C), solar heating (SH), *Trichoderma harzianum* (TH), and SH + TH.

In this experiment the emergence of bean seedlings in the soils previously exposed to solar heating was more uniform and occurred 1–2 days earlier than that of beans sown in the infested control soils.

Control of soilborne diseases in potatoes—field experiment 1978. General trends in this experiment were similar to those of the previous year but the differences were significant only in some cases (Table 2). The combined treatments were more effective in controlling the diseases and there was a trend towards higher yields than in the controls or those that received other treatments. Incidence of Verticillium wilt was significantly reduced by solar heating or solar heating combined with *T. harzianum*. The latter treatment was the only one that significantly reduced disease caused by *S. rolfsii*. Except for *T. harzianum* alone, all treatments reduced *R. solani* disease on bean seedlings. In this experiment a single irrigation was used for moistening the soil under polyethylene mulch (instead of a drip system). This facilitated the use of this technique, as was also shown in another study (4).

Control of *S. rolfsii* by physical, chemical, and biological means. Plastic net bags containing *S. rolfsii* sclerotia and buried in the soil at depths of 5 and 20 cm in various plots in the 1977 experiment, were recovered after 19 and 40 days and their viability was determined. Results (Table 3) show that at a depth of 5 cm all treatments killed the sclerotia of the pathogen (76–100%). At a depth of 20 cm, solar heating for 40 days, methyl bromide and metam-sodium also were effective (72–80% killed) but solar heating for only 19 days was not effective.

Integrated control of *S. rolfsii*, by combining physical and biological means, was tested under controlled conditions in the greenhouse. Soils, artificially infested with *S. rolfsii* sclerotia, were heated at 25, 47, and 50 C for 2 hr on four successive days, sown with beans (with and without the addition of *T. harzianum*) and maintained in the greenhouse for determining disease control. Heating or applications of *T. harzianum* partially controlled the disease, but a combination of both treatments was the most

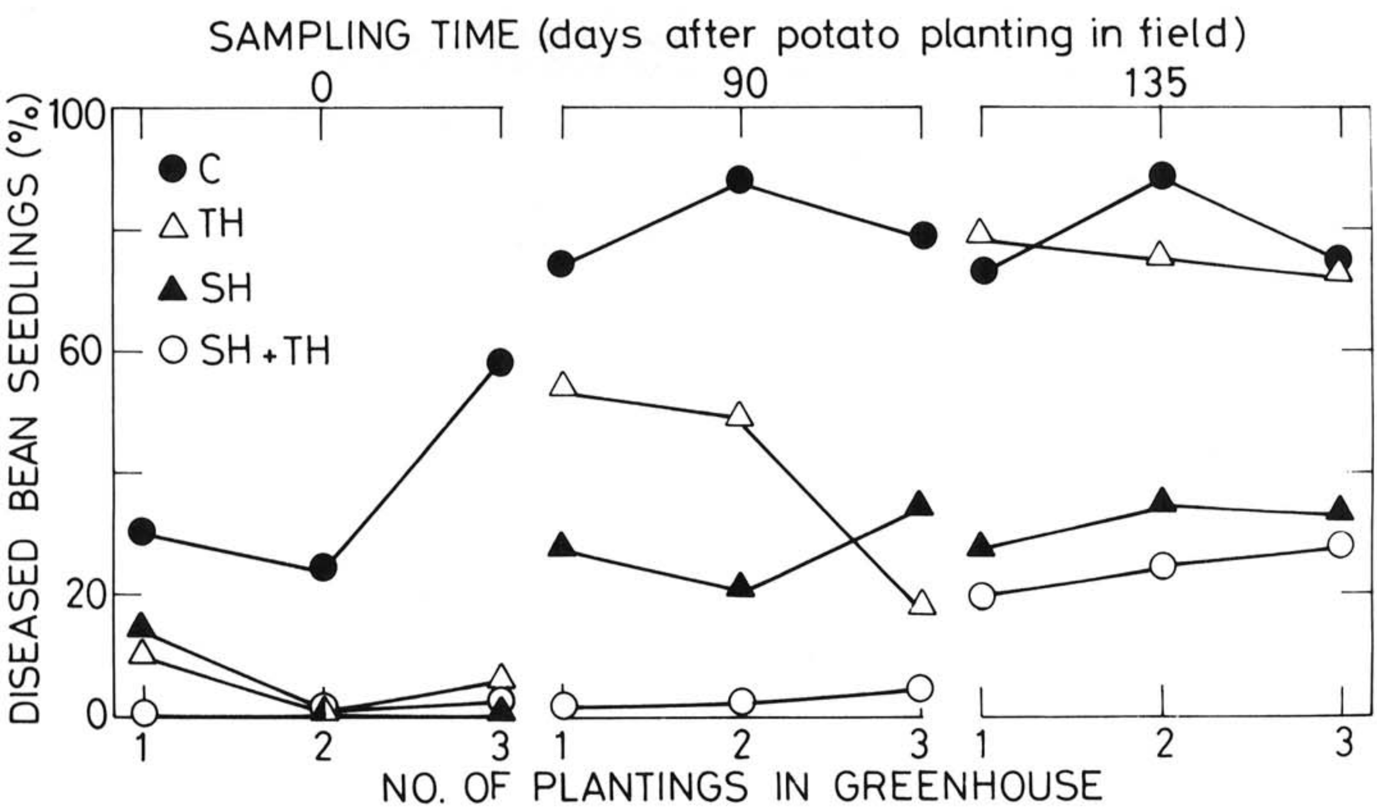

Fig. 2. Effect of repeated planting of beans in the soil under greenhouse conditions, on the incidence of *Rhizoctonia solani* disease. Soil samples were taken from plots in a field experiment at 0, 90, and 135 days after planting with potatoes. The treatments in the field were: Infested control (C), solar heating (SH), *Trichoderma harzianum* (TH), and SH + TH. Plantings were at 28-day intervals.

TABLE 2. Effect of soil treatments and *Trichoderma harzianum* (TH) on diseases and yield of potatoes[v]

Treatment	Dosage (kg/ha)	Diseased Plants[w] VD (No./100 m)	SR (No./100 m)	RS Greenhouse[x] (%)	RS Tubers (%)	Total Yield (kg/ha)
Infested control	...	47.7 a[y]	15.9 a	82 a	46.3 a	7,800 a
Ammonia[z]	2,000	33.7 ab	10.0 ab	57 b	34.3 a	7,176 a
TH	600	53.9 a	13.5 ab	77 ab	27.0 a	8,762 a
Methyl bromide (MB)	500	33.3 ab	6.7 ab	34 c	50.3 a	5,954 a
MB + TH	500 + 600	36.8 ab	3.4 ab	6 d	41.0 a	11,648 a
Solar heating (SH)	...	17.1 b	9.5 ab	0 d	29.3 a	6,682 a
SH + TH	0 + 600	20.1 b	0 b	1 d	26.3 a	10,412 a

[v] Field experiment, 1978, in northern Israel.
[w] VD = *Verticillium dahliae*, SR = *Sclerotium rolfsii*, RS = *Rhizoctonia solani*.
[x] Soil samples from the field plots were brought to the greenhouse and sown with bean seeds. Percentage of diseased plants was determined after 21 days.
[y] Numbers in each column followed by a common letter are not significantly different ($P = 0.05$).
[z] Dosage in liters per hectare.

TABLE 3. Effect of soil treatments on the killing of *Sclerotium rolfsii* sclerotia buried in the soil

Soil Treatment	Time of burial (days)	Mortality of sclerotia 5 cm (%)	20 cm (%)
Infested control	19	1 b[z]	1 b
Solar heating	19	100 a	25 b
Infested control	40	9 b	13 b
Solar heating	40	100 a	80 a
Methyl bromide (350 kg/ha)	40	76 a	72 a
Methyl bromide (500 kg/ha)	40	79 a	79 a
Metam-sodium (300 L/ha)	40	99 a	77 a

[z] Numbers in each column followed by a common letter are not significantly different ($P = 0.05$).

TABLE 4. Effect of soil heating and *Trichoderma harzianum* (TH) on the incidence of bean disease caused by *Sclerotium rolfsii*, determined 24 days after planting. Soil was mixed with the sclerotia of the pathogen (0.1 g/kg) before heating

Temperature (C)	TH inoculum concentration (g/kg) 0	2	4
25	93 a[z]	62 b	58 b
47	37 bc	9 de	4 e
50	17 cd	4 e	0 e

[z] Numbers in each column followed by a common letter are not significantly different ($P = 0.05$).

effective (Table 4), resulting in an almost complete disease control (90–100%).

In another experiment, the biocontrol efficiency of *T. harzianum* (at 2 g/kg) was tested in soil from either an untreated or a previously solar-heated field. *S. rolfsii* sclerotia, at concentrations of 0.05–0.4 g/kg soil were added to these soils, beans were sown in them, and they were maintained in the greenhouse to test for disease control. Biological control was more efficient with the previously heated soil; eg, at inoculum concentration of 0.25 g/kg, *S. rolfsii* disease reduction by *T. harzianum* in the untreated and solar heated soils was 50 and 90%, respectively.

DISCUSSION

The simultaneous control of all soilborne diseases of potatoes presents certain limitations and difficulties: the pathogens vary largely in biology and methods of control, only relatively inexpensive methods can be used with this crop, and it is necessary to protect the crop for the whole season in order to control tuber diseases. Moreover, environmental considerations and facility of application should be taken into account. Solar heating of the soil and fumigation with methyl bromide were distinguished by their broad spectrum of pathogen control. Combining these treatments with a biocontrol agent either in the field (Tables 1, 2, and Fig. 1) or in the greenhouse (Fig. 2 and Table 4) further improved their efficiency. An integrated control, which denotes the rational use of all available control measures, should be considered especially with a crop which is attacked simultaneously by numerous types and kinds of pathogens (7). Combining solar heating and *T. harzianum* had a long-term effect; the buildup of *R. solani* inoculum potential was slowed both in the field (Fig. 1) and under greenhouse conditions (Fig. 2). The synergistic phenomenon involved in the approach of an integrated control; ie, a pathogen weakened by sublethal doses, might be controlled more efficiently and for longer periods by antagonists, has already been shown and discussed (2,5,7,15,16). In such a situation, the populations of antagonists may increase since the weakened or dead cells of the pathogen serve as an enrichment medium. Though certain treatments increased the potato yield in our experiments, this was statistically significant only with methyl bromide and only in one experiment. This may be due to the large variation in the fields. In the present experiments the total yield was low, apparently due to the short period of plant growth because of climatic conditions. Further tests in fields where higher yields of potatoes are obtained are necessary in order to determine the economic benefit of the various treatments.

Solar heating of the soil may involve both a physical and biological control due to various mechanisms (8). Both such phenomena probably occurred in the present study. Populations of *Trichoderma* increased in the solar-heated plots. Incidence of disease caused by *R. solani* remained low throughout the season (Table 1)—apparently due to a shift in the biological equilibrium in the soil in favor of the antagonists. Such a shift in the solar-heated soil may explain the reduction in inoculum potential in the field (Fig. 1), the slow-down of inoculum buildup (Fig. 2), and the more efficient control when this treatment is combined with *T. harzianum*. Sclerotia of *S. rolfsii* were more vulnerable to soil microorganisms when subjected to sublethal heat (*unpublished data*) as was the case with conidia of *Fusarium* (8).

In a field experiment where the soil was highly infested with *V. dahliae* and *P. thornei*, solar heating significantly increased potato yield by 6,500 kg/ha (4). In the potato crop, both the pathogens and the soil and climatic conditions under which this crop is grown are diverse. It appears, therefore, that economic and environmentally safe methods for control can be achieved upon finding the optimal combination of all available control methods.

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