

SOLARIZATION OF GREENHOUSE SOIL FOR PREVENTION OF PYTHIUM ROOT ROT IN ORGANICALLY GROWN CUCUMBER

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SUMMARY

Soil solarization in greenhouse tests was carried out in 1995-1997 by covering pre-irrigated soil with transparent plastic sheets to prevent root rot caused by the *Pythium* species in organically grown cucumber, where *P. ultimum* was the main cause of root rot. Densities of naturally occurring *Pythium* in the upper 10 cm soil layer were determined before, during and after solarization. *Pythium* was reduced considerably after four weeks in experiments 1 and 2 and after two weeks in experiment 3. *Pythium* was not recorded in solarized plots, but was still present in non-solarized plots. The mean of daily maximum soil temperatures measured 10 cm below soil surface was 38-43°C in solarized plots compared to 31-37°C in non-solarized plots during the first 14 days of the experimental period. Pythium root rot was negligible 13 weeks after solarization of the soil, whereas serious disease symptoms were seen on plants grown in non-solarized soil. This investigation showed that solarization in the summertime could reduce Pythium root rot, even in the temperate climate of Denmark.

RIASSUNTO

LA SOLARIZZAZIONE DEL TERRENO IN SERRA PER LA PREVENZIONE DEL MARCIUME RADICALE CAUSATO DA *PYTHIUM* IN COLTURE BIOLOGICHE DI CETRIOLO. Esperimenti di solarizzazione del terreno sono stati eseguiti in serra nel periodo 1995-1997 coprendo terreno preirrigato con fogli plastici trasparenti per prevenire il marciume radicale da *Pythium* in colture biologiche di cetriolo, dove *P. ultimum* era la principale causa di marciume radicale. La densità della presenza naturale di *Pythium* nello strato superiore del terreno di 10 cm è stata determinata prima, durante e dopo la solarizzazione. *Pythium* è stato ridotto considerevolmente dopo quattro settimane nell'esperimento 1 e 2 e dopo due setti-

mane nell'esperimento 3. *Pythium* non è stato osservato nelle parcelle solarizzate ma era ancora presente in quelle non solarizzate. La media delle temperature massime giornaliere del suolo misurate a 10 cm sotto la superficie del suolo era di 38-43°C nelle parcelle solarizzate in confronto a 31-37°C di quelle non solarizzate durante i primi 14 giorni del periodo di sperimentazione. La moria delle piantine era trascurabile 13 settimane dopo la solarizzazione del terreno, mentre gravi sintomi della malattia erano osservati su piante cresciute in terreno non solarizzato. Questo studio ha mostrato che la solarizzazione durante l'estate potrebbe ridurre il marciume causato da *Pythium*, anche nel clima temperato della Danimarca.

Key words: *Cucumis sativus*, cucumber, Pythium root rot, *Pythium ultimum*, solarization, soil disinfestation, solar heating, soilborne disease.

INTRODUCTION

Many *Pythium* spp. are aggressive root pathogens and cause damping-off and root rot in older plants, resulting in plant death and reduced yield. Pathogenic *Pythium* spp. are unspecialized parasites that survive in soil on dead plant material and are primary colonizers of soils free of other micro-organisms, e.g. as a result of heat or fungicide treatment (Stasz and Martin, 1988). *P. ultimum* Trow is a common cause of root rot in greenhouse grown cucumber (Kröber, 1985; Thinggaard, 1994, unpublished).

Soil disinfestation carried out by chemical treatment or steaming is the traditional method for controlling harmful soilborne organisms in greenhouses. These methods are expensive and disturb the biological equilibrium in the soil ecosystem, eradicating not only the pathogens but also beneficial soil micro-organisms (Baker, 1962; Bollen, 1974). A non-hazardous method of soil disinfestation is solarization, which is based on trapping solar heat in the summer months by covering the moistened soil with transparent polyethylene sheets to increase soil temperature (Katan *et al.*, 1976). Solar-

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ization does not denude the soil of micro-organisms, as the survival rate of bacteria is higher than that of fungi, and many pathogenic fungi are likely to be killed at lower temperatures than saprophytic fungi (Baker, 1962; Bollen, 1969). The *Oomycetes* proved to be very sensitive in a soil pasteurising experiment (Bollen, 1969) and *Pythium* and *Phytophthora* were easily eliminated by soil steaming at 45-50°C for 30 min (Tjamos, 1992). It is important to keep non-pathogenic micro-organisms in the soil, because they compete with the pathogens for substrates, nutrients and space, thus preventing the reestablishment of pathogens (Kaewruang *et al.*, 1989).

Solarization was developed for soil in open fields in Israel with subtropic and tropical climates, where sufficiently high temperatures can easily be achieved (Katan *et al.*, 1976; Katan, 1981; Katan and DeVay, 1991). In the temperate zone of Japan, solarization has been found to be effective in the control of diseases in greenhouses (Kodama and Fukui, 1982; Horiuchi, 1991).

In greenhouse soils, solarization has reduced or controlled diseases in cucumber and tomatoes caused by the pathogenic fungus *Pythium* (Sarraf and Farah, 1989), but very little information about the efficiency and possibility of solarization in greenhouses in temperate climates is available.

The advantages are that solarization does not eradicate all soil micro-organisms, it is easy to apply, is less expensive than other soil disinfestation methods, is non-chemical, reduces many different pathogens, nematodes and weeds, and may have a long-term effect on some pathogens, such as *Fusarium* (Katan *et al.*, 1983) and *Verticillium* (Blanco-López *et al.*, 1992).

In a Danish greenhouse nursery with organically grown cucumber, problems with root rot caused by *Pythium* spp. were observed in 1994. Consequently, it was decided to try solarization as a measure to prevent *Pythium* root rot. The main questions were, whether sufficiently high temperatures could be achieved during the solarization period, and how long the treatment period should be to get an effective reduction of *Pythium* density. Therefore, three experiments were carried out in 1995-1997, and, in contrast to earlier experiments where soil samples were taken only before and after the experiment, samples were taken weekly or every second week during the treatment periods, in order to follow the reduction of *Pythium* populations.

MATERIALS AND METHODS

Identification and pathogenicity of *Pythium*

Identification. *Pythium* spp. were isolated from roots

and soil samples on *Pythium* selective agar medium (PSA) (Jeffers and Martin, 1986). The roots were surface disinfected in 70% ethanol for 20 s before placing on Petri dishes, and for isolation from soil samples a soil dilution plate method was used (Dhingra and Sinclair, 1985). *Pythium* spp. were isolated before and during three solarization experiments in two greenhouse sections. *Pythium* isolates from greenhouse sections A and B were grouped according to smooth or ornamented oogonia and according to their growth rate and pattern on corn meal agar (CMA, Difco, USA). One isolate from each group isolated from greenhouse section A was identified using the keys of Plaats-Niterink (1981) and Kröber (1985).

Pathogenicity test. A culture plate method developed by Altier and Thies (1995) was used to test the groups of *Pythium* isolates for pathogenicity on germinating cucumber seeds. The mean pathogenicity of one to three *Pythium* isolates from each group, was determined on a scale from 1 to 5. The severity of *Pythium* damping-off on germinating seeds and seedlings was described by a five class rating scale for individual seedlings: 1 = healthy seedling, 2 = primary root tip necrotic but firm, 3 = primary root tip rotted and soft, 4 = dead seedling, 5 = dead seed. Scale values indicate 1-2 = not pathogenic; 3 = partially pathogenic and 4-5 = highly pathogenic.

Greenhouse experiments

Greenhouse site. The growing medium in the 450 m² greenhouse sections of glass was clay soil with cow manure compost, and the soil was naturally infested with *Pythium*. Cucumber plants were grown on 15-20 cm raised beds, 100 cm broad, and watered with an irrigation system consisting of perforated plastic tubes. Three week-old cucumber seedlings were transplanted in February in 12 rows and replaced with new plants in mid-summer. Solarization experiments were carried out in between the two plantings, and the harvest period ended in October.

Experiment 1 (exp. 1) was carried out in greenhouse section A, exp. 2 and 3 in section B. In exp. 1, plots were prepared one week after removal of plants, without ploughing the soil, while in exp. 2 and 3, the soil was prepared in raised beds. All plots in exp. 3 were placed in areas solarized the year before, because the whole greenhouse section except for the control plots was solarized in exp. 2.

Experimental design. Each of the three experiments comprised two treatments, with and without solarization. Prior to solarization, the soil was moistened to

field capacity measured by tensiometers (Tensio-technic, Germany), and covered with 0.05 mm transparent polyethylene sheets (Garta A/S, Denmark). The sheets were buried along the edges to prevent air flow and to keep the soil moist. Bare soil without solarization treatment served as control. The greenhouse remained closed during the weeks of solarization except when soil samples were taken. The effect of treatment on soil populations of *Pythium* spp. was investigated within three replicate plots of 2 x 4 m in exp. 1, 1.6 x 5 m in exp. 2, and 2 x 2 m in exp. 3 for each treatment. In exp. 1, all six plots were evenly distributed in the greenhouse section, to represent the whole area, with the solarization plot and the control plot placed next to each other. The rest of the greenhouse section was without solarization. In exp. 2, the whole greenhouse section B was solarized except for two rows. In exp. 3, one fourth of section B was solarized and one fourth was not, the rest was steamed before the start of the experiment. In exp. 2 and 3, the three plots in the solarized rows were placed in such a way that 'border effect' was avoided. The three control plots were placed in rows without solarization. The duration of solarization was 8, 4, or 2 weeks, beginning in July, June, and May for exps. 1, 2, or 3, respectively.

Collection of soil samples. Soil samples of 1 litre were randomly collected at intervals of one or two weeks from the same place in each of the six plots. From each plot, three soil samples were taken from the upper 10 cm soil layer, before covering with polyethylene sheets, during, and at the end of the experimental period.

Determination of Pythium density. Quantitative estimations of *Pythium* were carried out twice for each soil sample by means of a soil dilution plate method: 0.5 ml of soil suspension was plated in Petri dishes with PSA using the drop technique (Dhingra and Sinclair, 1985). Plates were incubated for 2-3 days in darkness at about 22°C before the numbers of colony-forming units of *Pythium* per g dry soil (cfu g⁻¹ soil) were counted.

Temperature measurements. During solarization, soil temperature was recorded continuously 10 cm below the soil surface with two or three temperature sensors (Priva, The Netherlands) per treatment. Air temperature was measured every hour by a temperature sensor placed in the middle of the greenhouse (Priva, The Netherlands). Outside climate data were obtained from the Danish Meteorological Institute.

Transplanting. In exp. 2, the first transplanting was carried out immediately after removal of polyethylene

sheets. Twelve-three week-old cucumber plants, grown in peat free of *Pythium*, were planted as bait for *Pythium*, with plants evenly distributed in six plots and two treatments (6 plants per treatment). Root samples were taken with tweezers, in the upper 10 cm of soil, 10-20 cm from the stem of each bait plant, both on the day of planting and after 2, 5, 8, 11, and 13 weeks. Plants were pulled up after 13 weeks and disease incidence on roots described visually. The soil was left fallow over the winter, and new compost was worked into it in November before the second transplanting of 12 bait plants in March, 30 weeks after solarization. Root samples were taken on day of planting and 2, 5, 7 and 12 weeks after planting. Plants were pulled up after 12 weeks of growth and disease incidences described.

Pythium incidence on roots was investigated by placing three pieces of root samples, about 0.5 cm long and surface disinfected in 70% ethanol for 20 s, in Petri dishes with PSA, which were then incubated for 2 days in darkness at about 22°C before the incidence of *Pythium* spp. was recorded.

Soil samples were collected after the first transplanting in exp. 2., and samples were collected 2, 5, 8, 11, and 13 weeks after planting in the same way as during solarization.

Vertical soil samples for every 10 cm from 10 to 70 cm below the soil surface in solarization treated soil and in control soil were collected too. The samples were taken with an auger (1.8 cm in diameter), 8 and 11 weeks after the first transplanting, each sample was a mix of nine sub-samples. Quantitative estimations of *Pythium* in soil samples were carried out in the same way as for soil samples collected during solarization.

Statistical analyses. Solarization experiments consisted of two treatments (solarization and control) with three replications (plots) each. *Pythium* density during solarization was assumed to show Poisson distribution, and data were analysed by the Genmod procedure (SAS Institute, 1993) testing the effect of treatments and time of sampling on natural *Pythium* density.

RESULTS

Identification and pathogenicity of *Pythium*. Five *Pythium* groups were isolated and identified as follows: (i) *P. ultimum* Trow var. *ultimum*, with globose oogonia, sporangia missing, no special growth pattern, and mean growth rate of 48 mm 24 h⁻¹; (ii) *P. oligandrum* Drechsler with ornamented oogonia, sporangial complexes consisting of one or more subglobose elements with connecting filamentous parts, no special growth

pattern, and mean growth rate of 34 mm 24 h⁻¹; (iii) *Pythium* 'group G' [sensu Plaats-Niterink (1981)], with globose oogonia and sporangia, radial growth pattern, and mean growth rate of 25 mm 24 h⁻¹; (iv) *Pythium* sp. unidentified, with globule oogonia and sporangia, chrysanthemum growth pattern, and mean growth rate of 25 mm 24 h⁻¹; (v) *P. salpingophorum* Drechsler with globose oogonia, sporangia missing, radial growth pattern, and growth rate of 35 mm 24 h⁻¹.

The three major groups, 1-3, were isolated from soil and roots in both greenhouse sections. *Pythium* 'group G' could not be identified to species (Centraalbureau voor Schimmelcultures, Baarns, the Netherlands). Group 4 and 5 were isolated a few times from the soil only, *Pythium* sp. unidentified was isolated in both greenhouse sections, and *P. salpingophorum* was isolated from greenhouse section A only.

The pathogenicity on germinating seeds of the three major *Pythium* groups was as follows: *P. ultimum* var. *ultimum* was highly pathogenic, with a maximum scale value of 5, *Pythium* 'group G' was highly pathogenic too, with a scale value of 4.5, and *P. oligandrum* was non-pathogenic, with a scale value of 2. Pathogenicity test of the two minor groups showed that *P. salpingophorum* was highly pathogenic, with a scale value of 4.5, and isolates of the unidentified *Pythium* sp. were only partially pathogenic, with a scale value of 2.5.

Greenhouse experiments

Determination of Pythium density. Prior to experiments, *Pythium* density was 377 cfu g⁻¹ soil in exp. 1, 692 in exp. 2, and 27 in exp. 3 (Fig. 1). Solarization reduced these *Pythium* levels radically after 2 weeks and no *Pythium* could be recorded after 4 weeks in exp. 1 and 2, and after 1 week in exp. 3. A significantly faster rate of reduction was found for solarized plots than for control plots (*P* < 0.0001) in exp. 1 and 2. In exp. 3, with a low inoculum density, the reduction was not significant.

The average water content in solarized soil samples was almost stable and ranged from 33 to 37%, 35 to 39% and 38 to 42% for the three experiments respectively. In the controls, the water content of soil samples decreased during the solarization period, from 32 to 12% in exp. 1, from 29 to 21% in exp. 2, and in exp. 3 it increased a little from 33 to 40%.

Temperature measurements. In Table 1A, the max. and mean 1) of daily soil temp., 2) of soil temp. increase between solarization and control soil, and 3) of daily air temp. in the closed greenhouse sections during the first 14 days of experiments, are shown. In

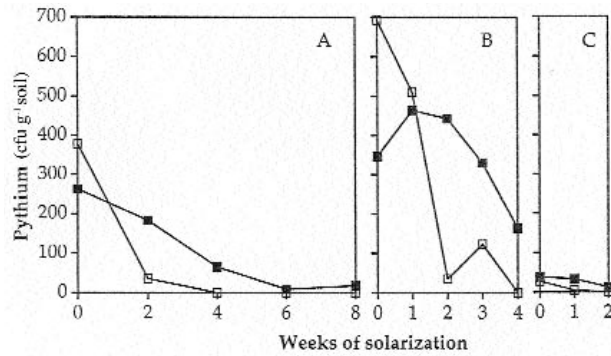


Fig. 1. Determination of *Pythium* density in soil samples taken in the upper 10 cm during solarization in experiment 1 (A), 2 (B) and 3 (C). Time 0 is the start of the experiment (just before covering with polyethylene sheets). Means of three solarized plots and three control plots. Difference between rates of decrease in solarized and control soil was significant for exp. 1 and 2 (*P* < 0.0001) but not for exp. 3 (*P* < 0.99). □ = solarization ■ = control.

Table 1. A. Maximum and mean, 1) of daily maximum temperatures of soil, 2) of soil temperature increases and 3) of daily air temperatures, in the closed greenhouse sections during the first 14 days of solarization experiments 1, 2 and 3. Soil temperatures as the mean of three temperature sensors per recording (every hour) 10 cm below soil surface are shown. (in exp. 3 only two sensors were used).

Experiment	Temp. (°C)					
	1	2	3	1	2	3
Soil temp.	Max.			Mean of daily max.		
Control	37	35	42	34	31	37
Solarization	44	45	50	41	38	43
Increase	8	10	8	7	7	7
Air temp.	48	51	58*	42	41	49*

* air temp. recordings for 12 days

Table 1. B. Maximum, minimum, and mean of outside air temperatures and summed sunshine hours for the local region for experiments 1, 2 and 3 during solarization periods. Climatic data were obtained from the Danish Meteorological Institute.

Experiment	Climatic data		
	1	2	3
Air temp. (°C)			
Maximum	30	22	24
Minimum	6	5	1
Mean	18	12	15
Sunshine hours	529/8 weeks	137/4 weeks	141/2 weeks
Sunshine hours	106/2 weeks	49/2 weeks	141/2 weeks

order to compare the long exps. 1 and 2 with the short exp. 3, only temperature measurements during the first 14 days of the solarization period are shown. Soil temperatures 10 cm below soil surface reached a maximum value after 25 days in exp. 1, after 7 days in exp. 2, and after 12 days in exp. 3. Climatic data for the local region of this work during the first two weeks of the solarization periods, are given in Table 1B. The weather during the whole experimental period in 1995 (exp. 1) was hot and sunny, in 1996 (exp. 2) it was more cloudy and cooler, and in 1997 (exp. 3) it was a sunny period.

Transplanting. At the end of the first transplanting (exp. 2), plants from the solarized plots had hardly any symptoms of root rot, while plants from control plots showed serious symptoms of *Pythium* root rot. In the second transplanting, all plants from solarized plots and control plots showed some symptoms of *Pythium* root rot and both white and brown roots were seen 12 weeks after planting. Brown and rotted parts on the lower 10 cm of stems of all 6 control plants were observed but only on one of the six plants grown in solarized plots.

Up to 5 weeks after the first transplanting, *Pythium* was not isolated from any plants in solarized plots, after which the number of plants with *Pythium* increased, and after 13 weeks, all plants were infected. In the control plots, *Pythium* had been isolated from all 6 plants as early as after 5 weeks (Table 2). In the second transplanting, *Pythium* was isolated in almost equal densities from plants in soil with and without solarization. After 5 weeks, *Pythium* was isolated from 4 and 5 plants out of 6 from solarized soil and control soil respectively (Table 2). *P. ultimum* was the dominating species isolated from the root samples in the first and second transplanting.

Pythium density (cfu g⁻¹ soil) in the upper 10 cm soil during the 13 weeks of first transplanting is shown in Table 2. The density of *Pythium* was 72-92% lower in solarized plots than in the control plots during the first period of transplanting. Max. *Pythium* density was 77 cfu g⁻¹ soil in solarized soil and 752 cfu g⁻¹ soil in the control soil (Table 2) decreasing as time progressed. Fig. 2 shows the vertical *Pythium* densities in six soil samples taken from 0 to 70 cm below the soil surface. The highest density of *Pythium* was found between 0 and 30 cm below the soil surface and the density decreased to lower levels 40-70 cm below the soil surface.

Table 2. Experiment 2: first transplanting (immediately after solarization) and second transplanting (30 weeks after solarization), each with 6 plants per treatment. Number of plants with *Pythium* isolated from the roots, and average *Pythium* density in the soil, average of three plots.

Treatment	Weeks after planting	Plants with <i>Pythium</i>	<i>Pythium</i> cfu g ⁻¹ soil
First transplanting¹			
Solarization	2	0	61
	5	0	75
	8	3	77
	11	5	13
	13	6	37
Control	2	4	752
	5	6	595
	8	6	273
	11	6	145
	13	6	171
Second transplanting²			
Solarization	2	1	–
	5	4	–
	7	4	–
	12	5	–
Control	2	2	–
	5	5	–
	7	5	–
	12	6	–

¹ *Pythium* density before first transplanting was 0 cfu g⁻¹ soil for solarization plots and 164 cfu g⁻¹ soil for control plots.

² Compost and soil mixed before second transplanting.

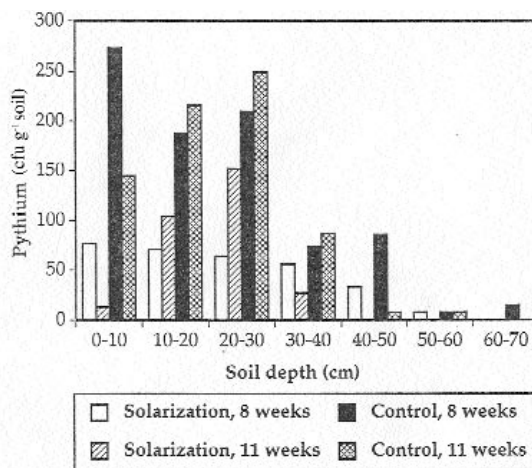


Fig. 2. Experiment 2: density of *Pythium* spp., 0 to 70 cm below the soil surface. The soil samples were taken from one solarized plot and one control plot, 8 and 11 weeks after first transplanting (21 and 24 weeks after solarization). One soil sample consists of nine sub-samples.

DISCUSSION

The solarization experiments were effective in reducing *Pythium* at high as well as low *Pythium* inoculum densities. The reduction of the pathogen can be ascribed to the effect of thermal killing by moist heat, which reduced the inoculum potential to such an extent that the disease was reduced. Irrigation of the soil just before solarization increased the thermal sensitivity of the pathogen (Baker, 1962) and caused an improvement of heat conduction in the soil (Mahrer, 1979).

In practice, populations of soilborne fungal pathogens are greatly reduced at temperatures of 40-50°C. For the upper 30 cm layers, exposure time usually ranges from minutes to hours for the higher temperatures and up to days for the lower ones. Since temperatures in the deeper soil layers are lower than in the upper ones, the treatment period should be sufficiently extended, usually 4 weeks or longer, to achieve pathogen control at all desired depths (Katan, 1981). In our experiments, maximum temperatures of 44-50°C were easily achieved in the upper 10 cm soil during the first 14 days of solarization, when *Pythium* was greatly reduced. Similarly, Lebanon soil temperatures reached 41°C and 47°C during solarization for 49 and 43 days, respectively, in plastic tunnel greenhouses (Sarraf and Farah, 1989).

Use of solarization in greenhouses in a temperate climate depends on and varies with the meteorological conditions during the season and the latitude. Outside max. air temperatures in exp. 2 were 22°C and thereby lower than the 27°C and 24°C in exp. 1 and exp. 3 respectively, but even so, there was a significant effect of solarization in exp. 2. Sunshine hours for the first two weeks of solarization were also lower in exp. 2. This shows that even a cloudy and cool summer can elevate air temperatures up to 51°C in the closed greenhouse and make solarization effective. Closing the greenhouse is important for raising temperatures in solarization experiments (Garibaldi and Tamietti, 1983).

Solarization in temperate climates may even have an effect in the field, which was seen in the moderate temperature conditions of south-eastern Idaho, having a mean max. air temp. between 26-33°C, when max. soil temp. reached 41°C at 15 cm soil depth (Davis and Sorensen, 1986).

During solarization, biological control of the pathogen may be achieved and may continue after solarization, because of the survival of and changes in the populations of soil micro-organisms (Stapleton and DeVay, 1984). A pathogen may be weakened by sublethal heating, which increases its vulnerability to the suppressive activity of soil micro-organisms, leading to a reduc-

tion in disease incidence (Katan *et al.*, 1992).

The first transplanting carried out immediately after solarization showed that the reduction in *Pythium* in the upper 10 cm soil, even though *Pythium* was found in deeper layers, was sufficient to reduce disease incidence in cucumber. Reduction of the pathogen, mainly in the upper 10 cm soil, is in contrast to traditional soil disinfestation methods, where the primary objective is to eradicate the pathogen completely, also in deeper layers, but at the same time, most of the soil micro-organisms disappear (Kreutzer, 1965). The first transplanting showed a clear reduction in *Pythium* root rot symptoms after 13 weeks and delayed the incidence of *Pythium* on the roots. No effect of the solarization in exp. 2 could be found 32 weeks after treatment. For practical use in temperate climates, solarization can only be effective in greenhouses in the summertime, when the temperature is highest. This is in accordance with solarization experiments carried out in northern Italy, where solarization had an effect on disease in greenhouses but not in open fields (Garibaldi and Tamietti, 1983; Garibaldi, 1987). Because of a short growing season in the northern hemisphere, no more than two weeks of solarization can be accepted by the growers. If the treatment has to be effective after a shorter time or has to reach deeper soil layers, supplemental heating, *e.g.* by application of heating pipes, must be added. The use of aerated steam at 50-60°C is also a possibility (Dawson and Johnson, 1965). To be efficient and long-lasting, solarization should be regularly applied in the early stage of soil infestation (Pullman *et al.*, 1981). As part of a strategy, solarization should be used together with other preventive methods against soilborne diseases, *e.g.* combined with the use of resistant varieties, grafting, integration of biological control agents, and amendment of organic materials to the soil. Compost combined with solarization gave pathogen control and higher temperatures (Gamliel and Stableton, 1993).

It can be concluded that the use of soil solarization can reduce *Pythium* root rot in greenhouse cucumber in temperate climates under optimal weather conditions in the summertime.

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