BARD

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**Clinical (Major) and Subclinical (Minor) Root-
Infecting Pathogens in Plant Growth Substrates,
and Integrated Strategies for their Control**

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plant growth substrates, and integrated strategies for their control**

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ABSTRACT

In intensive agriculture, harmful soilborne biotic agents, cause severe damage. These include both typical soilborne (clinical) major pathogens which destroy plants (e.g. *Fusarium* and *Phytophthora* pathogens), and subclinical ("minor") pathogens (e.g. *Olpidium* and *Pythium*). The latter cause growth retardation and yield decline. The objectives of this study were: (1) To study the behavior of clinical (major) and subclinical (minor) pathogens in plant growth substrate, with emphasis on zoosporic fungi, such as *Pythium*, *Olpidium* and *Polymyxa*. (2) To study the interaction between subclinical pathogens and plants, and those aspects of *Pythium* biology which are relevant to these systems. (3) To adopt a holistic-integrated approach for control that includes both eradivative and protective measures, based on a knowledge of the pathogens' biology.

Zoospores were demonstrated as the primary, if not the sole propagule, responsible for pathogen spread in a recirculating hydroponic cultural system, as verified with *P. aphanidermatum* and *Phytophthora capsici*. *P. aphanidermatum*, in contrast to *P. capsici*, can also spread by hyphae from plant-to-plant. Synthetic surfactants, when added to the recirculating nutrient solutions provided 100% control of root rot of peppers by these fungi without any detrimental effects on plant growth or yield. A bacterium which produced a biosurfactant was proved as efficacious as synthetic surfactants in the control of zoosporic plant pathogens in the recirculating hydroponic cultural system. The biosurfactant was identified as a rhamnolipid. *Olpidium* and *Polymyxa* are widespread and were determined as subclinical pathogens since they cause growth retardation but not plant mortality. *Pythium* can induce both phenomena and is an occasional subclinical pathogen. Physiological and ultrastructural studies of the interaction between *Olpidium* and melon plants showed that this pathogen is not destructive but affects root hairs, respiration and plant nutrition. The infected roots constitute an amplified sink competing with the shoots and eventually leading to growth retardation. Space solarization, by solar heating of the greenhouse, is effective in the sanitation of the greenhouse from residual inoculum and should be used as a component in disease management, along with other strategies.

OBJECTIVES

Objectives of the Original Research

1. To study the behavior of clinical (major) and subclinical (minor) pathogens in plant growth substrates, with emphasis on zoosporic fungi, such as *Pythium*, *Olpidium* and *Polymyxa*.
2. To study the interaction between subclinical pathogens and plants, and those aspects of biology of zoosporic fungi, which are relevant to these systems.
3. To adopt a holistic-integrated approach for control that includes both eradication and protective measures, based on the knowledge of pathogens' biology.

I. STUDIES IN THE USA

Title: Clinical (major) and subclinical (minor) root-infecting pathogens in plant growth substrates, and integrated strategies for their control.

Final Report 1997. Arizona. Principle investigator in Arizona: Michael E. Stanghellini.

Objective 1. Determination of the significance of zoospore production and dispersal in rockwool.

Objective 2. Whole plant studies in the greenhouse. Specific objectives were to (A) determine an effective method of inoculation and evaluate the efficacy of (B) an antagonistic bacterium and (C) surfactants in control of root rot caused by *Pythium aphanidermatum*.

Results relative to the above objectives:

1. We demonstrated, for the first time, that zoospores are the primary, if not sole, propagule responsible for pathogen spread in a recirculating hydroponic cultural system. This conclusion was verified in two plant/host interaction systems: *Pythium aphanidermatum* vs. cucumbers and *Phytophthora capsici* vs. peppers. In both systems, zoospores were identified as the sole propagule responsible for spread via the recirculating nutrient solution. However, our results also demonstrated that *Pythium aphanidermatum*, in contrast to *P. capsici*, can also spread by hyphae from plant-to-plant if plants are growing in the same rockwool block (publications 1,2,3).

2. We demonstrated that synthetic surfactants, when added to the recirculating nutrient solutions provided 100% control of root rot of peppers and cucumbers caused by *P. capsici* and *P. aphanidermatum*, respectively, without any detrimental effects on plant growth or yield (publications 1 & 2). We are now evaluating the use of synthetic surfactants for use in open-field agriculture against root-infecting as well as foliar zoosporic plant pathogens.

3. We evaluated the efficacy of a commercial bacterium (*Pseudomonas cepacia*) as a biological agent for control of *P. aphanidermatum* root rot of cucumbers in a hydroponic cultural system. Repeated greenhouse trials demonstrated that no control was realized despite the fact that the bacterium effectively colonized cucumber roots in the cultural system. However, we did discover a bacterium (a fluorescent *Pseudomonas* species) which produces a biosurfactant which proved as efficacious as synthetic surfactants in the control of zoosporic plant pathogens in a recirculating hydroponic cultural system. The biosurfactant was identified as a rhamnolipid. Purified rhamnolipids were subsequently demonstrated to rupture the plasmamembrane of zoospores, which resulted in death of the latter infectious propagules and control of disease caused by zoosporic plant pathogens. Preliminary results were published as a Feature Article in Plant Disease (publication #).

Referred Publications and abstracts resulting from the BARD project no. IS-2155-92. Reprints (2) of each refereed publication are included in this report.

1. Stanghellini, M.E., S.L. Rasmussen, D.H. Kim, and P.A. Rorabaugh. 1996. Efficacy of nonionic surfactants in the control of zoospore spread of *Pythium aphanidermatum* in a recirculating hydroponic system. *Plant Dis.* 80:422-428.

2. Rasmussen, S.L., M.E. Stanghellini, D.H. Kim, and P.A. Rorabaugh. 1995. Efficacy of surfactants in the control of zoosporic root-infecting fungi. *Phytopathology* 85:1167.

3. Stanghellini, M.E., D.H. Kim, S.L. Rasmussen and P.A. Rorabaugh, 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. *Plant Dis.* 80:1113-1116.

4. Stanghellini, M.E. 1997. Inert ingredients - are they really so? *Phytoparasitica* (in press).

5. Stanghellini, M.E., and Miller, R.M. 1997. Biosurfactants: their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Dis.* 81:4-12.

II. STUDIES IN ISRAEL

1. Occurrence of clinical (major) and subclinical (minor) soilborne pathogens

In intensive agriculture, both in greenhouses and in open fields, harmful soilborne biotic agents, upon accidental introduction, may become a limiting factor. These include both typical soilborne (clinical) pathogens which destroy plants (e.g. *Fusarium* and *Phytophthora* pathogens), and subclinical ("minor") pathogens (e.g. *Olpidium* and *Pythium*). The latter cause growth retardation and yield decline, similar to the phenomenon of "soil sickness", common in soil-grown monocultures. Most of the researches concerning soilborne pathogens concentrate on major pathogens, whereas subclinical pathogens have been much less studied. Our preliminary results indicated that under certain conditions, *Olpidium*, *Polymyxa* and *Pythium* are potential subclinical (non-destructive) pathogens (see more details in section 2 below). We have, therefore, concentrated on these fungi and studied their occurrence at various sites in Israel, especially since data on these fungi as subclinical pathogens is scarce in general and non-existing in Israel.

Procedures: Plants grown on soil or on artificial growth substrates (media) were collected from various sites in Israel (open fields and greenhouses) with emphasis on crops grown in monoculture or on plants suspected to suffer from growth retardation. The plants were examined microscopically for biotrophic fungi. In addition, root pieces were incubated on selective media and the fungi obtained were recorded. Inoculation and other tests were carried out to determine the possible role of these fungi in growth retardation and yield decline.

Major findings:

- a) *Olpidium*, *Polymyxa* and *Pythium* spp. were detected in roots of Gypsophila, muskmelon and cucumbers - plants growing in monoculture. *Pythium* spp. were also isolated from tomato plants in monoculture on artificial growth substrates, such as tuff (volcanic ash), rockwool or compost. Typical examples of distribution of these fungi are given in Tables 1 and 2. The following species were identified:
 - i) Biotrophic fungi, subclinical agents: *Olpidium radicale*, *O. brassicae* (needs final species verification) and *Polymyxa*.

- ii) Species of *Pythium*: *P. aphanidermatum*, *P. sylvaticum*, *P. proecandrum* and *P. irregulare*. These fungi can act both as clinical pathogens (at high inoculum concentration) and subclinical, as will be described below.
- b) Samples of growth substrates from greenhouses, in which the crops were in monoculture were collected. They were brought to the greenhouse, mixed with noninfested Rehovot soil and planted with melon or cucumber and their growth in this mixture was compared to that of plants in noninfested Rehovot soil. Results (Table 3) show that dry shoot weight was significantly reduced by planting the plants in infested diluted medium. The roots of plants in the infested soil were colonized with *Olpidium* and *Pythium*.
- c) Growth retardation (by 65%) of melon plants was induced by growing them in Rehovot noninfested soil mixed with infected roots of plants from monoculture. Concomitantly, the roots of plants in the infested soil were heavily colonized with *Olpidium* and *Pythium*.
- d) Inoculation tests: Many inoculation tests with zoospore of *Olpidium*, *Polymyxa* or *Pythium* were carried out with Gypsophila, melon and cucumber plants, and with tomatoes inoculated with *P. aphanidermatum*. We found that in all cases, *Olpidium* and *Polymyxa* caused growth retardation, without inducing plant mortality. We therefore consider them as typical subclinical ("minor", non-destructive) pathogens. *Pythium* at low concentrations, or at less severe conditions, caused low or no plant mortality (but caused growth retardation) while at high inoculum concentrations it caused high mortality incidence and severe plant growth retardation. We therefore consider *Pythium* as occasional or conditional subclinical pathogen. Typical examples are given in Tables 4-7. Further details on plant-microbe interactions and on the interactions between microorganisms will be given below.
- e) Co-inoculation with two subclinical pathogens resulted in a synergistic effect, namely a more severe mortality, as compared with each microorganism alone (Tables 8, 9). Additional experiments showed a corresponding detrimental effect on plant biomass resulting from co-inoculation.
- f) Clinical (major) pathogens: Continuous or frequent cropping of crops results in a build up of populations of both subclinical and clinical (major pathogens), especially in soilless culture. Of the latter, we should mention the following:

- i. Fusarium crown and root rot of tomatoes caused by *Fusarium oxysporum* f. sp. *Radicis-lycopersici*. This disease became destructive in soilless culture since the conidia produced on the stems are dispersed by the water in soilless culture. Moreover, resistant cultivars are not yet used in Israel. This disease is also destructive in soil. We found that this disease has a polycyclic nature, namely it is spread during the growth season.
- ii. Fusarium wilt diseases, especially Fusarium wilt in melon caused by *F. oxysporum* f. sp. *melonis*. We found in Israel all the known physiologic races of the pathogen, therefore the use of resistant cultivars has a limited value. Fusarium wilt of cucumber was also detected but is still on a limited scale. Fusarium wilt of tomatoes (caused by *F. oxysporum* f. sp. *lycopersici*) became rare due to the use of cultivars which are resistant to races 1 and 2 of the pathogen and the absence of race 3. However, the recent introduction of cherry tomatoes, which lack genes for resistance to Fusarium wilt, resulted in reappearance of this disease. Moreover, we found that this pathogen produces aerial macroconidia on the stems. These propagules are dispersed in the air, as has been proved by spore trapping - this is the first record of aerial dissemination of this soilborne pathogen. We found a similar phenomenon with *F. oxysporum* f. sp. *basilici*. This is much related to sanitation of the greenhouse structure (see section 3 below).
- iii. We found wilt and collapse of mature (2-4 months old) tomato plants in soilless culture, which was caused by *Pythium* spp., especially *P. aphinedermatum* - the disease is not known in Israel in soil culture, neither in greenhouse nor in the open field. This disease may become a threat in soilless culture. A similar phenomenon was observed in rockwool in Arizona by M. Stanghellini, the American partner, and the implications in each country have been discussed in depth by the researchers in this project, with other researchers and with extension specialists.
- iv. Root rots caused by *Phytophthora* spp. in ornamentals, e.g. Gypsophila in soilless culture. These diseases have been rare in Israel, so far. It is anticipated that their incidence will further increase in soilless culture in the future.

In planning disease management programs, the development of clinical (major) soilborne pathogens, both in soilless and in soil culture (along with subclinical pathogens) should be considered.

In conclusion of this part of the research:

Yield decline is a common phenomenon in monoculture, in both soil and container media (soilless culture). *Gypsophila* and melon yields were significantly reduced, under commercial monoculture conditions in soil or tuff medium (volcanic ash). Yield reduction was observed after several seasons in soils, or after one or more seasons in soilless culture. Growth retardation was reproduced in *Gypsophila* and melon seedlings planted in monocultured soil or tuff medium in pots. Subclinical (minor) pathogens are widespread in soils and soilless culture. They colonize cortex cells and juvenile root tissues. Plants infected with minor pathogens did not exhibit destructive symptoms, although growth and yield were adversely affected. *Pythium* spp., the biotrophs *Olpidium brassicae* and *Polymyxa betae* were observed. In addition, *Olpidium radicale* was found in melon. These fungi along with major pathogens are widespread, they play a role in yield decline, their incidence is increasing in soilless culture and have the potential to become a limiting factor in greenhouses.

Co-infection of plants with subclinical pathogens may result in a synergistic and detrimental effect.

2. Interactions between subclinical (minor) pathogens and plants

“Minor” (deleterious) pathogens which cause subclinical diseases; they do not cause complete destruction of the plant. They invade roots, meristematic tissues, rootlets and root hair, reducing root activity and plant vigor. Although their interaction with the host is mild, it results in growth retardation and yield reduction. They are typical to monoculture crops in soil and in soilless culture. The microorganisms involved include bacteria, actinomycetes and fungi, such as *Pythium*, *Penicillium* and the zoosporic fungi *Olpidium* and *Polymyxa* which are usually known as viral vectors. Since our results indicated that the biotrophic fungi *Olpidium* and *Polymyxa* cause plant growth retardation (but not plant mortality) in the absence of viruses, we regarded them as typical subclinical pathogens and studied their interactions (especially of *Olpidium*) with plants. Our studies (described below), indeed

confirmed that these organisms are not destructive and their interaction with plants is mild.

Light microscopic and scanning electron microscopic examinations revealed damage to root hairs of the inoculated melons. Light microscopic examination of melon roots inoculated with *O. radicle* revealed the presence of thalli in the epidermal cells including root hairs and in the cortical cells. Sporangia, with exit tubes through which the zoospores are liberated, were observed five days after inoculation. Mature resting spores were observed about one month after inoculation. Transmission electron microscopic studies of inoculated roots showed that thalli of *Olpidium* developed in very close contact with the host cell cytoplasm. Host-cell walls appeared intact and no changes in the integrity of the host cells were observed until 14 days after inoculation. One month after inoculation, the host cells appeared somewhat damaged, however, intact host-cell organelles could still be observed.

Physiological parameters of the *O. radicle*-melon, host parasite relationships were studied. Respiration rates of infected melon roots were increased by 30-100% (on fresh root basis) 3-30 days after inoculation. Root respiration, computed on dry tissue basis, was not affected 3 weeks after inoculation or later, in accordance with the increased content of dry matter in infected roots. Protein concentration in the dry roots of infected plants increased from the third day; respiration rate, based on protein level was, hence, not affected by *Olpidium* infection. Respiration, photosynthesis and chlorophyll content of leaves were not affected by infection.

In melon seedlings grown in nutrient solutions, phosphorous and nitrogen concentrations in the infected roots were higher than in non-infected plants, while potassium and sulphur concentrations were lower. Mineral concentration in the leaves were not affected by infection. When the nutrient solution was diluted to 0.5 and 0.25 of its full concentration, the relative growth retardation, caused by *Olpidium* infection became more severe.

Specific uptake (uptake/unit root weight) of potassium in infected plants was significantly less than in the non-infected ones, whereas that of phosphorous was significantly higher. Relative translocation (% of total in the shoot) of potassium was higher in infected plants whereas relative phosphorous translocation was lower. Growth retardation and reduction in biomass of infected plants, could be the result of several mechanisms: (1) damage to root cells and root hairs of rootlets, which are

responsible for uptake of nutrients and water; (2) reduced rates of potassium uptake; (3) retention of phosphorous in the roots; (4) decrease in minerals content of the plant (root and shoot); (5) increased root respiration and a concomitant increase in carbohydrate utilization. Thus, the infected roots constitute an amplified sink, competing with the shoot, and eventually leading to growth retardation. It is concluded that *Olpidium* does not cause stunting or growth inhibition of the shoot directly. The initial damage is caused to the roots it consequently results in reduced shoot growth and yield decline.

Figures and pictures are presented at the end of this part (Figs. 1-9).

3. Aspects of disease management

Intensive agriculture in greenhouses, usually in monoculture, results in a buildup of inocula of plant pathogens, especially soilborne. Large amounts of infected plant residues persist in the soil and in the greenhouse structure. These become a source of primary inoculum for the next crop. The soilborne inocula are usually eliminated by soil disinfestation. In contrast, the inocula adhering to the greenhouse structure are usually not effectively controlled, due to technical difficulties involved in reaching these inocula at all sites. We, therefore, need various management methods to eliminate or control all sources of inocula. Complementary studies on disease management have also been done by the American partner (please see the relevant section).

Space solarization: Closing the greenhouse during the period between crops, which is usually done in the summer time in Israel, raises air temperatures to 60°C and higher (space solarization). However, this heating occurs under conditions, which is less effective than under wet conditions (Fig. 10). The effectiveness of space solarization in the sanitation of the greenhouse from pathogen was assessed by hanging small nylon nets containing inocula of various types in the greenhouse, and recording rate of survival in time, during space solarization.

The inoculum of *Clavibacter michiganese* was the most sensitive and that of *Fusarium oxysporum* f. sp. *radicis-lycopersici* was at least sensitive to space solarization; *Pythium* sp. and *Sclerotium rolfsii* were intermediate (Fig. 11; 11a). Wetting the greenhouse structure cooled the greenhouse (Fig. 12) and did not

improve pathogen control (Fig. 13), since this resulted in a temporary cooling due to evaporation. We examined various models which relate level of heating to rate of pathogen control. The difficulty in developing this type of predictive models is that natural solar heating involves fluctuating temperatures, as opposed to constant temperatures which prevail under artificial heating. We obtained good results by computing the accumulated degree - hour above certain temperatures and relating them to the corresponding values of pathogen control (Figs. 14 and 15). A second approach is to consider heat mortality as a first order reaction. This model is followed successfully in food processing for heat disinfestation at constant temperatures. First results obtained in 1996 are promising. The validity of this model for space solarization will be further studied in 1997.

A study on the prewetting of pathogen propagules on their sensitivity to space solarization is described below (see also Table 10).

Solarization of artificial growth substrates: Inocula of soilborne pathogen survive in the soil as well as in the artificial growth substrates. We found that solarization is very effective in controlling *Pythium*, *Rhizoctonia* and *Fusarium* in soil and soilless culture. Yield increase was evident also in the second crop after solarization. Both yield and quality were improved, thus confirming previous results. Space solarization must be combined with solarization of the soilborne inoculum in order to ensure a full disease control.

THE EFFECT OF A WETTING PRE-TREATMENT ON THERMAL DEATH OF *SCLEROTIUM ROLFSII* AND *FUSARIUM OXYSPORUM* PROPAGULES.

SUMMARY

Solar heating of soil and air in closed greenhouse is an additional approach for improving soil- and air-borne pests control in regions with high temperature and intense solar radiation. Space solarization, namely, disinfecting the greenhouse space and structure, by closing and by heating it by solar radiation, is carried out during summer time, thereby raising air temperatures up to 55-65 °C and killing the plant pests by dry heat. To achieve thermal inactivation of micro-organisms by dry heat, higher temperatures and longer exposure times are required than those for moist heat. Therefore, a detailed information on the effect of prewetting of the pathogen propagules on their thermal inactivation may improve the efficiency of dry heating treatment. It was found that prewetting treatments decreased sensitivity to heat of multicellular propagules such as sclerotia of *Sclerotium rolfsii*, macroconidia of *Fusarium oxysporum* f. spp.: *radicis-lycopersici*, *lycopersici* and *basilici*. The opposite (increased heat sensitivity) was observed with unicellular propagules, such as chlamydospores of *F. oxysporum* f. spp. *radicis-lycopersici* and *melonis*. Alternating drying and wetting pre-treatment increased heat sensitivity of sclerotia of *Sclerotium rolfsii*. Cycloheximide and low temperatures suppressed heat tolerance of prewetted sclerotia of *S. rolfsii*, indicating a possible involvement of a metabolic process (e. g. heat shock proteins). Scanning electron microscope (SEM) observations revealed that heating increased the frequency of cracks in the rind of *S. rolfsii* sclerotia. Prewetting treatments reduced significantly the frequency of surface cracks on heated sclerotia. The results of this study indicate that an improved greenhouse sanitation by space solarization can be achieved under proper conditions.

Key words: soil and space solarization, thermotherapy, solar heating, thermal inactivation.

Table 1: Infection of roots of muskmelon and cucumber plants with *Olpidium* and *Pythium* spp. in commercial fields (F) and greenhouses (Gh), tested at the end of the growth season.

Site	Crop F/Gh	Medium ¹	Treatment ²	<i>Olpidium</i> ³	<i>Pythium</i> ⁴
Ein-tamar X 2 ⁵	Muskmelon Gh	TP	SH	2	0
Ein-tamar X 2	Muskmelon Gh	TP	UN	4.5	Pa; Pp
Ein-tamar	Muskmelon F	S	MB	2	0
Ein-tamar	Muskmelon F	S	SH	3	0
Ein-tamar	Muskmelon F	S	UN	5	Pa; Pp
Hazeva X 5	Muskmelon F	S	MB	4	Pa; Pp
Hazeva	Muskmelon Gh	T	UN	4	Pa; Pp
Hazeva	Muskmelon Gh	R	UN	0	0
Almog X 3	Muskmelon F	S	MB	4	nt
Neve-yaar	Cucumber Gh	TP	UN	3	P.sp
Hazav	Cucumber Gh	T	UN	4	Pa; P.sp
Hazav	Cucumber Gh	T	MB	2	0
Hazav	Cucumber Gh	T	V	3	Pa; P.sp
Kefar-menahem	Cucumber Gh	T	UN	1	Pa; Ps; Pu
Yashresh	Cucumber Gh	S	UN	3	nt
Tamra	Cucumber F	HS	UN	1	P.sp

¹ S= Sandy soil; TP= Mixture of tuff and organic matter as artificial growth substrate; R= Rockwool; T= Tuff medium; HS= Heavy soil.

² Un= Untreated monoculture soil or medium; SH= Soil solarization; MB= methyl bromide treatment; V= vapam treatment

³ A 6-grade index in which 0= no infection and 5= more than 80% of observed roots infected with *Olpidium*.

⁴ *Pythium* spp. : Pa = *P. aphanidermatum*; Pp = *P. paroecandrum*;

Pu = *P. ultimum*; Ps = *P. sylvaticum*; P.sp = *Pythium* sp.; 0 = no *Pythium* infection; nt = not tested.

⁵ X= Similar results was observed from X different fields at the same site.

Table 2. Presence of potential subclinical pathogens in roots of *Gypsophila* plants collected from commercial greenhouses in Israel or on roots of wild *Gypsophila* growing in non-agricultural soil (NA).

Site	Root colonization with		
	<i>Olpidium</i>	<i>Polymyxa</i>	<i>Pythium</i>
1. Soil			
Kefar Warburg	- ^a	+	+
Kidron	-	-	+
Kefar Pines	+	+	+
Beorotayim	+	+	+
Olesh - 1 ^b	-	+	+
Olesh - 2	+	+	-
Zohar	-	+	+
Hogla	-	-	-
2. Artificial substrate			
Orot	+	+	+
Shekef	+	+	+
Gea	+	-	+
Geva Carmel	+	+	+
Kefar Warburg	+	+	-
Sedeh Warburg	-	+	-
3. NA - 1^c			
NA - 2	+	-	-
NA - 2	-	-	-

^a + = present; - = absent.

^bTwo different plots at this site.

^cTwo different sites.

Table 2: Shoot weight and colonization of roots of muskmelon and cucumber plants by *Olpidium* and *Pythium* spp., 30 days after planting in the indicated soil or growth substrate.

Exp. no.	Crop	Medium ¹	Storage ²	Dry shoot weight (g/plant)	<i>Olpidium</i> ³	<i>Pythium</i> ⁴
1	Melon	s/tp	0	0.28 b	5.0	Pa; Pp
		s/tcomp	0	0.13 b	5.0	Pa; Pp
		s	0	0.85 a	0.5	0
2	Melon	s/tcomp	3	0.15 b	5.0	Pa; Pp
		s	3	0.38 a	0	0
3	Melon	s/tcomp	5	0.25 b	5.0	Ps
		s	5	0.46 a	0	0
	Cucumber	s/tcomp	5	0.88 a	1.5	Ps
		s	5	0.96 a	0	0
4	Cucumber	t	0	1.47 b	3	Ps
		t(mb)	0	2.13 a	0	0
		t (ot)	0	1.87 a	0	0
		s/t	0	0.98 b	4.6	Ps

¹ s=Rehovot sandy soil, served as a noninfested control ; s/tp= Mixture of 4:1 Rehovot sandy soil, tuff and peat mixture in which muskmelon grew in monoculture; s/tcomp= Mixture of 4:1 Rehovot sandy soil with mixture of tuff and compost in which muskmelon grew in monoculture; t= cucumber monocultured tuff medium ; t(MB)= t after methyl bromide treatment; t(ot)= t after one hour treatment in otoclave; s/t Mixture of 4:1 Rehovot sandy soil with t.

² Number of years in storage. The medium was stored dry, under shade condition, until used.

³ A 6 - grade index in which 0= no infection and 5= more than 80% of observed roots infected with *Olpidium*.

⁴ Pa = *Pythium aphanidermatum*; Pp = *P. paroecandrum*; Ps = *P.sp.* ; 0= no infection with *Pythium*.

5 Numbers followed by different letters in each experiment indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$)

Table 4. Effect of inoculation with Pythium aphanidermatum on growth of muskmelon seedlings and on % seedling mortality (SM).

Inoculation concentration cfu/g	<u>Dry weight, mg</u>		SM
	Root	Shoot	%
0	106 a	139 a	0
0.7	64 b	144 a	10
1.4	51 b	107 b	10
14.0	74 b	125 ab	30
70.0	54 b	120 b	40
110.0	50 b	120 b	70

Table 5. Effect of inoculation with *Pythium* spp. on growth of *Gypsophila* seedlings^a.

<i>Pythium</i> sp.	Inoculum concentration CFU/g soil ^b	Shoot dry weight g/plant
Uninoculated	0	1.53 a ^c
<i>P. paroecandrum</i>	5	1.32 a
<i>P. paroecandrum</i>	10	1.00 a
<i>P. paroecandrum</i>	50	0.68 b
<i>P. aphanidermatum</i>	8	1.45 a
<i>P. aphanidermatum</i>	16	1.42 a
<i>P. aphanidermatum</i>	80	1.18 ab
<i>P. aphanidermatum</i>	120	0.75 b

^aNo plant mortality was observed at all inoculum concentrations.

^bCFU = colony forming units.

^cValues with a different letter are significantly different ($P = 0.05$).

Table 6. Effect of inoculation with *Olpidium* on growth of melon plants^a.

Parameter	Uninoculated	Inoculated
Shoot fresh weight - g/plant	10.33*	5.99
Shoot dry weight - g/plant	0.70*	0.33
Root fresh weight - g/plant	3.28	2.28
Root dry weight - g/plant	0.13	0.12
Dry weight (%) in root	4.02*	5.70

^aNo plant mortality was observed.

^bAn asterisk indicates a significant difference ($P = 0.05$).

Table 7. Effect of inoculation with Polymyxa on growth of gypsophila seedlings.

Parameter	Noninoculated	Inoculated*
Shoot, fresh weight (g)	9.87	6.90
Root, fresh weight (g)	6.11	3.74
Shoot, dry weight (g)	1.32	0.96
Root, dry weight (g)	1.38	0.60

* With each parameter, inoculated were significantly different ($P = 0.05$) from noninoculated.

Table 8. Effect of inoculation with Olpidium radicale (OR) Pythium aphanidermatur (PA), alone or combined on mortality (SM) of muskmelon seedlings.

Pathogen	SM %
None	0
PA	20
OR	0
PA+OR	85

Table 9. Effect of inoculation of gypsophila with *Pythium ophanidermatum*, (Pa) and *Olpidium radicale* (Or) alone or combined, on mortality of melon plants

Treatment No.	Inoculation	Mortality (%)
1	Noninoculated	0
2	Pa - 25 CFU/g soil ^a	20
3	Pa - 100 CFU/g	30
4	Or - 200,000 zoospores/ml	0
5	Or - 100,000 zoospores/ml	0
6	Treatment 2 + treatment 4	85 ^b
7	Treatment 2 + treatment 5	65 ^b
8	Treatment 3 + treatment 4	85 ^b
9	Treatment 3 + treatment 5	65 ^b

^a CFU = colony forming units

^b Significantly different (P= 0.05) from each of the corresponding individual treatments

Table 10. Effect of prewetting length on heat sensitivity of propagules of four pathogenic fungi.

		Pathogens, survival (%)							
		<i>S. rolfii</i> sclerotia		<i>F.o. basilici</i> macroconidia		<i>F.o. radicis-lycopersici</i> chlamydospores		<i>F.o. melonis</i> chlamydospores	
Prewetting ¹ (hr)		No heat	Heat	No heat	Heat	No heat	Heat	No heat	Heat
0	100 ² Aa ³		29.5 Bc	100 Aa	15.8 Bbc	100 Aa	71.1 Ba	100 Aa	63.4 Ba
1	100 Aa		44.2 Bc	100 Aa	22.8 Bb	98.8 Aa	55.6 Bb	94.2 Aa	47.7 Ba
8	95.4 Aa		73.7 Bb	100 Aa	36.8 Bab	97.6 Aa	32.1 Bc	93.2 Aab	32.3 Bb
24	100 Aa		94.0 Aa	100 Aa	46.2 Ba	95.3 Aa	21.1 Bd	87.2 Ab	21.2 Bc

¹ Propagules were exposed to 0-24 hrs prewetting before subjected to wet heat for one hour at 41⁰ C (*Fusarium oxysporum* f. sp. *melonis*) 43⁰ C (*S. rolfii* and *F. oxysporum* f. sp. *basilici*) and 44⁰ C (*Fusarium oxysporum* f. sp. *radicis-lycopersici*).

² Numbers are survival percentage determined by comparison with propagules maintained at 25⁰ C.

³ Lower case letters within each column denote significant differences among prewetting treatments ($P \leq 0.05$). Upper case letters within each row, for each pathogen, denote significant differences between heat treatments ($P \leq 0.05$).

Fig. 1. Effect of inoculation with *Olpidium radicale* on protein content in roots of muskmelons. In all cases, inoculation had a significant difference ($P = 0.05$).

Fig. 2. Chlorophyll content (mg/g fresh weight) in the third and fourth leaf of melon plants, inoculated (infected) and noninfected, 34 days after inoculation. No significant differences between pairs of treatments.

Fig. 3. Respiration (CO₂ evolution) of melon roots inoculated with *Olpidium*, as compared to infected plants at days 3 to 30 after inoculation. dw = dry weight; fw - fresh weight.

Fig. 4. Rate of photosynthesis of melon plants, either infected (= inoculated with *Olpidium*) or noninfected, at various light intensities. No significant differences between treatments at each light intensity.

Fig. 5. Stomatal conductivity of melon plants, either infected (= inoculated with *Olpidium*) or noninfected, at various light intensities. No significant differences between treatments.

Fig. 6. Effect of inoculation with Olpidium radicale on mineral content in leaves and roots of muskmelon plants. Astriks denotes a significant difference ($P=0.05$) between inoculated and noninoculated plants.

Fig. 7. Relative translocation of P and specific uptake of K in melon plants, either infected (= inoculated with *Olpidium*) or noninfected at various relative concentrations of the mineral solution. Asterisks indicates a significant difference ($P=0.05$) within each pair of treatments. Numbers on each pair indicate infected/noninfected ratios.

Fig. 8. Scanning electron microscopy of melon roots, 7 days after inoculation with *Olpidium*. A. (magnification x 50): noninoculated plant roots; B-D (magnification x 50): inoculated plants; E,F (magnification x 250): noninoculated (E) and inoculated plant roots.

Light
Fig. 9. ~~Scanning electron~~ microscopy of melon rootlets. A,B - rootlets infected with *Polymyxa*; C. Thallus of *Olpidium radicale* in the host all; D. Sporangium of *Olpidium*; E. resting spores of *Olpidium* in the host cell; F. rootlets heavily colonized with *Olpidium*. Figs. A,F at magnification of 10 x 10, BCDE at magnification of 10 X 50.

Fig. 10 (upper figure). Effect of dry and wet heating on survival of *Fusarium oxysporum* f. sp. *radicis-lycopersici*.

Fig. 11. Effect of space solarization (by closing the greenhouse) on survival of soilborne pathogens in the greenhouse. CR = *Fusarium oxysporum* f.sp. *lycopersici*; C.MICH = *Clavibacter michiganense*.

Survival of FORL & S.rolfsii Space solar. - Rehovot 1995

11.a.
Fig. 11. Rate of pathogen control by space solarization. FORL= Fusarium oxysporum
f. sp. radicis-lycopersici.

Influence of 3 daily irrigations on temp. regime

Fig. 11 (lower figure). Air temperature in a cooled greenhouse without (dry) and with irrigation.

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Fig. 6. Survival of *Fusarium oxysporum* f. sp. *melonis* maintained for 0-20 days in a closed greenhouse without (dry) and with (wet) supplemental irrigation.

Exponential curves of Fusarium (F.o.r.l) Degree-Hours basis

Fig. 14. Relationships between level of heating by space solarization (expressed as accumulated degree - hours above 45 or 55°C) and mortality rate of *Fusarium oxysporum* f.sp. *radicis-lycopersici*.

Control of Rolfsii sclerotia Greenhouse space solar. - Rehovot 94

Fig. 15. Relationships between level of heating by space solarization (expressed as accumulated degree-hours above 50°C) and rate of mortality (transformed) of sclerotia of *Sclerotium rolfsii*.

COOPERATION

The cooperation regarding this project between the American and Israeli investigators started by writing the proposal, which took place in Arizona during J. Katan's visit there. J. Katan again visited M.E. Stanghellini in 1995 and M.E. Stanghellini visited J. Katan in 1996. The investigators also met on additional occasions, such as the meetings of the American Phytopathological Society. The investigations have been carried out in continuous contacts throughout the research period, exchanged results and publications and consulted each other. Some of the experiments were jointly planned. This cooperation yielded two planned projects: one on epidemiology, which was submitted and another project on sudden wilt of melons, to be submitted shortly. We are certain that our scientific relations will be long lasting.

RESEARCH ACHIEVEMENT

1. We demonstrated, for the first time, that zoospores are the primary, if not the sole propagule responsible for pathogen spread in a recirculating hydroponic cultural system. This conclusion was verified in two plant/host interaction systems: *Phythium aphanidermatum* vs. Cucumbers and *Phytophthora capsici* vs. Peppers. In both systems, zoospores were identified as the sole propagule responsible for spread via the recirculating nutrient solution. However, our results also demonstrated that *P. aphanidermatum*, in contrast to *P. capsici*, can also spread by hyphae from plant-to-plant if plants are growing in the same rockwool block (USA, publications 1, 2, 3).
2. We demonstrated that synthetic surfactants, when added to the recirculating nutrient solutions provided 100% control of root rot of peppers and cucumbers caused by *P. capsici* and *P. aphanidermatum*, respectively, without any detrimental effects on plant growth or yield (publications 1 & 2). We are now evaluating the use of synthetic surfactants for use in open-field agriculture against root-infecting as well as foliar zoosporic plant pathogens.
3. We evaluated the efficacy of a commercial bacterium (*Pseudomonas cepacia*) as a biological agent for control of *P. aphanidermatum* root rot of cucumbers in a

hydroponic cultural system. Repeated greenhouse trials demonstrated that no control was realized despite the fact that the bacterium effectively colonized cucumber roots in the cultural system. However, we did discover a bacterium (a fluorescent *Pseudomonas* species) which produces a biosurfactant which proved as efficacious as synthetic surfactants in the control of zoosporic plant pathogens in the recirculating hydroponic cultural system. The biosurfactant was identified as a rhamnolipid. Purified rhamnolipids were subsequently demonstrated to rupture the plasma membrane of zoospores, which resulted in death of the latter infectious propagules and control of disease caused by zoosporic plant pathogens. Preliminary results were published as a Feature Article in *Plant Disease* (USA publication 5).

4. We have shown the existence and the widespread of subclinical (minor) pathogens which infection results in plant growth retardation and yield decline but not in plant mortality. These pathogens include *Ovipodium* and *Polymyxa* and were associated with melon, cucumber and *Gypsophila*. *Pythium* spp. is an occasional subclinical pathogen: at low inoculum concentration it causes growth retardation and at high inoculum concentration it causes plant mortality.
5. Coinoculation of *Ovipodium* or *Polymyxa* with *Pythium* results in increased plant mortality and reduced plant growth.
6. Physiological and ultrastructural aspects of the interactions between the subclinical pathogen *Ovipodium* and melon plant were investigated. These studies included respiration and mineral nutrition aspects.
7. We have followed various approaches for the management of pathogens as stated above. In addition, we studied space solarization as a tool for the decontamination or sanitation of the greenhouse structure from residual inoculum. The experimental data were compared with predicted data based on models relating heating to mortality rates of pathogens.

CONCLUSIONS

Understanding the biology of the pathogen and the mode of its survival are the key factors in developing effective and environmentally acceptable disease management approaches. Zoospores are the sole propagule responsible for spread via the circulating

nutrient solution in zoosporic fungi. Hence, selected synthetic surfactants were very effective in such systems. On the other hand, residual inoculum on the greenhouse structure can be treated by space solarization. In addition, biocontrol agents have a potential as a component in integrated disease management programs. Both subclinical and clinical root pathogens should be considered in intensive agriculture, especially in soilless systems.

PUBLICATIONS

Referreed publications and abstracts resulting from the BARD project No. IS-2155-92. Reprints of each referreed publication are included in this report (the USA part).

1. Stanghellini, M.E., S.L. Rasmussen, D.H., Kim and P.A. Rorabaugh. 1996. Efficacy of nonionic surfactants in the control of zoospore root-infecting fungi. *Plant Dis.* 80:422-428.
2. Rasmussen, D.H., M.E. Stanghellini, D.H., Kim and P.A. Rorabaugh. 1995. Efficacy of surfactants in the control of zoospore root-infecting fungi. *Phytopathology* 85:1167.
3. Stanghellini, M.E., D.H., Kim, S.L. Rasmussen, and P.A. Rorabaugh. 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. *Plant Dis.* 80:1113-1116.
4. Stanghellini, M.E. 1997. Inert ingredients - are they really so? *Phytoparasitica* (in press).
5. Stanghellini, M.E. and R.M. Miller. 1997. Biosurfactants: their identity and potential efficacy in the biological control of zoospore plant pathogens. *Plant Dis.* 81:4-12.
6. Shlevin, E., J. Katan, Y. Mahrer and G. Kritzman. 1995. Space solarization for sanitation of plant pathogen in the greenhouse structure. *Phytopathology* 85:1209.
7. Primo, D. P., E. Shlevin, J. Katan and Y. Mahrer. 1997. Heat mortality of pathogens as affected by moisture during solarization. *Phytoparasitica* 25:251-252.
8. Katan, T., E. Shlevin and J. Katan. 1997. Airborne inoculum of *Fusarium oxysporum* f. sp. *lycopersici* in greenhouses of cherry tomatoes. *Phytopathology* (in press).