MEETING

ABSTRACTS OF PAPERS PRESENTED AT AN INTERNATIONAL WORKSHOP ON

MANAGEMENT OF SOILBORNE PATHOGENS

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Summary and Recommendations

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Management of soilborne plant pathogens, including plant parasitic nematodes, is one of the single greatest challenges facing modern agriculture worldwide. Virtually without exception, crops produce less when planted repeatedly in the same soil than when grown in rotation. This is primarily because the repeated exposure of the soil flora and fauna to roots, stems, and other tissues of the same plant species (or variety) enriches the soil for pathogenic fungi, bacteria, and nematodes with ability to infect and parasitize those tissues. In spite of this fact, the trend worldwide for all of the major crops and most of the specialty crops is towards shorter or no crop rotation as industries based on these crops become more specialized. Yield decline, soil fatigue, monoculture injury, replant problems, allelopathy, and soil sickness are among some of the terms used to describe an almost universal problem. In addition to lower yields and often poorer quality products, crops with diseased roots are more likely to leave water and nutrients unused in the soil and are less competitive with weeds.

The importance of soilborne pathogens in modern agricultural systems is made especially clear by the current rush worldwide to find alternatives to methyl bromide (MB) for preplant treatment of soils used to produce certain high-value horticultural crops. Yield increases of 200-300% were virtually universal for strawberries, tomatoes, and other crops in response to soil fumigation with MB alone or in combination with other fumigants starting some 30 years ago. Such yield increases in response to a single treatment remain without precedent to this day, and reveal that crop damage caused by soilborne plant pathogens, when left unmanaged, becomes widespread, chronic, and highly significant. Unfortunately, the release (or escape) of MB into the atmosphere affects the ozone layer, and for this reason the use of this fumigant is to be phased out by 2001, 2005, or 2015, depending on the country. All crops, and not just those with the market value to pay for soil fumigation, respond more or less the same to soil fumigation because all crops are subject to more or less the same relative amounts of chronic and acute damage from their soilborne pathogens, when grown without benefit of adequate crop rotation (or plant-free fallow). Furthermore, the principles and nonchemical options for disease management are the same for all crops, whether or not the crop is currently produced with the aid of MB. Practices such as burning cereal stubble or thorough tillage of fragile soils, done to control soilborne pathogens of cereals in many parts of the world, can also have major negative effects on the environment or natural resource base.

To address these questions and challenges, nearly 100 plant pathologists from 12 countries participated in the 5-day workshop on Management of Soilborne Plant Pathogens, held 1-5 March 1998 in Jerusalem, Israel, and funded by the U.S.–Israel Binational Agricultural Research and Development (BARD) fund and The Hebrew University of Jerusalem. The workshop started with a welcome reception on the evening of 1 March followed by three full days of sessions and, on 5 March, by an all-day tour that included morning visits to historical sites followed by lunch and an afternoon visit to view crop production at Kibbutz Sa'ad.

Each participant was asked in advance to submit an abstract of his or her contribution; these were made available in bound form at the Workshop and are published in this issue of *Phytoparasitica*. In addition, the participants discused research needs and priorities, and these are summarized below.

General priorities and strategies for management of soilborne pathogens

Research and education on management of soilborne pathogens without depending on MB or other environment- or resource-degrading methods should emphasize integration of:

- Cropping systems
- Innovative cultural practices
- Use of resistant or tolerant varieties
- Use of high-quality pathogen-free planting material
- Conservation or enhancement of pathogen-suppressive soils
- Introduction of microbial biocontrol agents
- Optimization of the disease-suppressing benefits of organic matter
- Use of physical and biological methods of soil disinfestation

Emphasis on basic research should include:

Developing a greater understanding of:

- the molecular and genetic mechanisms of plant defense against soilborne pathogens, as a means of improving the resistance or tolerance of crop plants and making greater use of induced resistance to soilborne pathogens;
- the molecular root–microbe, antagonist–pathogen, and root–antagonist–pathogen interactions, as a basis for new approaches to biological control of soilborne pathogens;
- the relationship between organic matter quality, composition, and content of soils and suppression of plant diseases;
- population dynamics of the species, subspecies, and biotypes of soilborne pathogens responsible for low yields, poor quality and short life of crops, when grown under intensive management and controlled when soils are fumigated or solar-heated; and
- the role of physical and chemical variables and their interactions in the ecology of soilborne pathogens as a means of making greater use of cultural and biological control.

Reaching an understanding of genetics, genomics, and evolutionary biology for a few representative soilborne pathogens and antagonists, as a means to predict and prevent or delay the emergence of new pathotypes of pathogens and improve the performance of biological control agents.

Emphasis on applied research and extension should include:

Increasing the effort on:

- breeding and release of agronomically and horticulturally acceptable cultivars of crop plants, including rootstocks, for resistance or tolerance to soilborne pathogens;
- use of agricultural and municipal wastes, composts, and other organic substrates as soil amendments for suppression or eradication of soilborne pathogens;
- cropping systems research and education involving plants, crops, crop sequences and agronomic practices for management of soilborne pathogens; and
- discovery, genetic improvement, and formulation techniques for delivery of microbial biocontrol agents to plants and soils for management of soilborne pathogens.

Developing more effective and efficient techniques and methodologies for screening plant and microbial germplasm for novel genotypes, genes or gene products for use in management of soilborne pathogens.

Developing and implementing novel and better approaches to solarization and other physical methods for soil disinfestation, to improve the efficiency and reduce the costs of these options to soil fumigation.

The following proposals were made at the end of the meeting:

1. To define and test under various conditions select packages or systems for management of soilborne pathogens. These packages (species and varieties of crop plants or biocontrol agents, soil treatment methods, etc.) should be the same in all participating countries.
2. To analyze and compare the obtained results.
3. To establish field demonstration plots for the farmers in each participating country.
4. To establish a permanent soilborne pathogens committee for the Mediterranean and Middle East countries; the first meeting of the group could be in Morocco.

Adoption, Adaptation and Implementation of Soil Solarization: Achievements and Difficulties

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Soil solarization (SoSo) is a nonchemical soil disinfestation method which harnesses solar energy for heating the soil. It involves hydrochemical processes leading to physical, chemical and biological changes in the soil, which take place during and even after the termination of solarization. Under the appropriate conditions, SoSo controls a variety of pathogens, weeds and arthropod pests. A long-term effect in disease control and enhancement of plant growth and yield has been observed frequently due to SoSo. SoSo is the only nonchemical means of pest management which, under appropriate conditions, is comparable to methyl bromide fumigation. Its major limitations are climate dependency and occupation of land for several weeks. Elucidation of the mechanisms involved in solarization will provide potential tools for its improvement. For example, understanding the weakening phenomenon enables a better determination of optimal combinations of SoSo with other control agents. Developing physical and biological models will enable a more reliable prediction of solarization effectiveness under various conditions. Physiological changes in plants growing in solarized soils have been studied only recently and they indicate the presence of signals in soil possibly affecting the root and the upper plant parts. SoSo can be improved in various ways. A very promising approach is its combination with other pest-management methods, such as organic amendments, which produce volatiles that accumulate under the plastic tarp. SoSo can be used for a variety of purposes in addition to soil disinfestation, *e.g.* production of healthy propagation material, especially transplants, and sanitation. Like most nonchemical methods, SoSo is not backed by commercial companies, and therefore its implementation and adoption by farmers have become bottlenecks. Although thousands of hectares are solarized every year in many countries, the potential for its use is much higher than that. Soilborne pathogens can be regarded as biopollutants. Therefore, a nonchemical soil disinfestation method should be considered as a remediation of agricultural soils from these biopollutants.

Novel Rotation Crops for the Management of Phytonematodes and Other Soilborne Plant Pathogens

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A number of tropical and subtropical plant species can be used as rotation crops to manage problems caused by nematodes and other soilborne plant pathogens. Among these antagonistic plants are legumes (*Aeschynomene americana, Cannavalia ensiformis, Cassia fasciculata, Crotalaria juncea, C. spectabilis, Indigophora hirsuta, Pueraria* spp., *Mucuna* spp.), forage grasses (*Chloris gayana, Digitaria decumbens, Panicum virgatum, Paspalum notatum*), and plants in several other families (*Ricinus communis, Sesamum indicum,* and some species in the Cruciferae and Compositae). The mode of action of these crops against nematodes is usually based on production of antihelminthic root exudates and the establishment of rhizosphere organisms favored by the exudates. Many species of bacteria and fungi in the rhizosphere of these plants are antagonistic to root-knot nematodes (*Meloidogyne* spp.) and other economically important phytonematodes. The concept of utilizing select rotation crops to manipulate the soil microflora and increase suppressiveness against specific pathogens can be tested with antagonistic plants. Soils with velvetbean (*Mucuna deeringiana*) are considerably more suppressive of root-knot and other soybean (*Glycine max)* nematodes than soils in soybean monoculture. Our results suggest that it is possible to design suppressive cropping systems to deal with problems caused by nematodes and other soilborne plant pathogens through the use of plants that stimulate microbial antagonism to the pathogens.

Soil Solarization, a Model for Introducing a New Technology into Traditional Agriculture

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Soilborne pathogens (SBP) threaten agricultural sustainability and production in Egypt (population 60 million) in the limited cultivated area (7.3 million acres), especially in smallholdings where the water management system is not efficient.

Soil solarization was first introduced in Egypt in 1981 to minimize the harmful effects of SBP. Data from solarization experiments and demonstration plots in farmers' fields showed effective control of soilborne fungi, nematodes, arthropods and weeds. Physical and chemical properties of the soil were improved, salinity was reduced and plant growth and crop yield were increased. Solarization was effective for seed-bed nurseries, tomatoes, onions, and in plastic-house crops. However, there are some constraints to the implementation of soil solarization on a large scale in Egypt, and possibly in other developing countries. (i) Proper extension is needed to convince the farmers who are following traditional practices. (ii) The best time for mulching in the open field is during June to August and in plastic houses from May to September (double heating). At that time, most of the available land is cultivated with summer crops. A few thousand acres are devoted to some vegetable crops, where soil solarization could be implemented. (iii) In the old land, most of the 4 million acres are cultivated intensively with various crops (1.9 crops per year) to obtain maximal yield. Hence, there is no time for solarization. (iv) Cost-benefit ratio: Crops in Egypt have a flexible marketing system depending upon supply and demand. Mulching 1 acre requires 300 kg of 60-μm-thick polyethylene sheets, at a cost of US$485. The average value per acre of the yield of some crops is: cotton, $1059; rice, $706; corn, $500; tomato, $2941; strawberry, $2058; and onion, $1764.

Soil solarization has a long-term effect in controlling several SBP and in increasing yield for two to six successive crops, and is less expensive than methyl bromide, which costs $1647 per acre. Combining soil solarization for only 20 days with methyl bromide (25 g/m^2) is effective, thereby shortening the period needed for solarization, while reducing the pesticide dosage.

Methods to Evaluate Organic Amendments for the Control of Soilborne Plant Pathogens

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Screening for efficacy of organic products to control soilborne pathogens involves complex interactions that require weeks or months to complete. Tests must be carried out in soil in order to include interactions with the soil microbial community. This complicates recovery and quantification of the target pathogen. We have used *Verticillium dahliae* Kleb. and *Streptomyces scabies* (Thaxter) Lambert & Loria as models for evaluating disease and inoculum reduction following application of organic amendments. *V. dahliae* is particularly suited since it is highly adapted for survival in soil.

The bioassay involves isolating microsclerotia (MS) of *V. dahliae* from cultures, sieving them to size, placing these into nylon mesh bags and then into soil. For field experiments the bags are glued to plastic marker stakes and lowered into soil. Bags are recovered and the MS are transferred onto a semi-selective agar. Germination and colony growth of the MS are scored 2 weeks after plating. We found that MS survived at least one year after burial in untreated soils. Comparison of the survival of MS in field and laboratory assays of identical treatments showed good agreement (r^2=0.82). We have tested a wide spectrum of organic materials including animal by-products, manures, and bio-solids added to soil in the laboratory and field. Quantities of the test material were mixed with soil from potato fields with a history of Verticillium wilt and Streptomyces scab. Typically, incorporation of products such as meat and bone meal killed MS within 7–10 days at 24°C and 50% water holding capacity. MS were also killed in the headspace, indicating the toxic agent(s) is volatile. Ammonia is most likely the volatile product accounting for the rapid death of MS. The MS assay also revealed that amendment efficacy is determined by factors such as pH, temperature and soil moisture. More than one mechanism is involved in the death of MS following incorporation of amendments. Where ammonia toxicity was not involved, MS died gradually between 2 and 6 weeks following amendment. Here the correlation with death of MS and disease protection was not clear, since plants may have been infected prior to the death of MS. Immediately following fumigation, populations of soil microorganisms are reduced dramatically. In contrast, amendments increased total numbers of fungi and bacteria by up to 1000 times, although numbers of *V. dahliae* and *S. scabies* declined. The efficacy of a single application of various organic materials for wilt control was evaluated in microplots and commercial fields over a 4-year period. The greatest benefits to yield were achieved in the second year after application but were no longer apparent by the fourth year. These results suggest that high-nitrogen-containing amendments can be used to control soilborne pathogens.

B: MANAGEMENT OF THE PHYSICAL-CHEMICAL ENVIRONMENT OF PATHOGENS IN SOIL

Management of Soilborne Pathogens of Protected Tomato in Morocco

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In Morocco, the intensification of protected tomato cultivation has exacerbated disease problems. Therefore, integrated soilborne diseases management (IDM) programs should be implemented. Proper agricultural practices improve plant growth and enhance disease control. Soil and water salinity increase the susceptibility of tomato plants, particularly to Fusarium and Verticillium wilts. Lowering the salinity level reduces the incidence and severity of these pathogens. Effective rotation is not feasible in protected tomato. Soil-less culture is efficient for disease control, but substrate infestation by some pathogens may occur if sanitation and fungicide control procedures are not applied properly. Resistant rootstocks provide excellent control of many tomato soilborne. This technique, is now widely used in Morocco and also in many other countries. Many tomato fungal and bacterial soilborne pathogens are seed-transmitted. The use of pathogen-free seeds is an important component of any tomato IDM program. Many tomato cultivars are resistant to various soilborne pathogens but not to all of them. The rise of new races, particularly of *Fusarium* and *Verticillium*, is a threat. Soil solarization controls many tomato pathogens, and solarization of tomato supports is successful in controlling pathogens such as *Didymella*. Methyl bromide (MB) is widely used in tomato. Other chemicals do not provide consistent control comparable to that of MB. In many countries various factors limit a wider application of IDM programs, the most important being the perceived complexity of the method, inadequate information, low cost of some pesticides, activities of the chemical companies, and a lack of knowledge about IDM. Thus, most of the tomato IDM components are not applied by the farmers who produce tomatoes for the local market; the farmers who produce tomatoes for export are more technically advanced.

Management of *Rhizoctonia solani* in Various Crops

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Rhizoctonia is a taxonomic complex form genus which includes a great number of different anastomosis groups (AGs), causing a variety of disease symptoms on many plant hosts. The diseases range from seed rot to pre- and post-emergence damping off, root and stem rots, storage organs (roots, tubers and bulbs) rots, foliar rots disseminated by airborne basidiospores, and fruit rot either by the airborne inoculum or by soil inoculum infecting fruit which is in contact with soil. The severe sheath blight disease of rice in paddies has a special pattern of sclerotial behavior. The sclerotia develop air bags which make them float and infect the rice plants at the water–air interface and cause severe losses. Crops such as sugar beet, soybean and bean are infected by the soilborne as well as by the airborne inoculum. Management of *Rhizoctonia* diseases varies according to the environmental conditions, the specific crops, the pathogens, and the type of diseases occurring in the crop. Different isolates of the various AGs have different susceptibilities to various fungicides. Therefore, the disease management is designed to suit the specific requirements of each crop.

The following practices are used for the control of *Rhizoctonia:* Cultural practices: Sanitation, soil pasteurization, soil solarization, free fallow season, tillage *vs* no tillage (plowing), practices favoring rapid emergence (escape), proper plant spacing (ventilation), pathogen-free propagation material, timing of irrigation – soil moisture, relative humidity, fertilization, green harvesting, crop rotation, resistant cultivars.

Biological control: Amendments, crop residues, various kinds of organic matter, chitin, suppressive composts, antagonistic fungi, (*Trichoderma, Gliocladium*, non-pathogenic *Rhizoctonia*), rhizosphere-antagonistic bacteria (*Bacillus subtilis*, fluorescent *Pseudomonas* spp., *Burkholderia cepacia*), induced resistance, transgenic plants with resistance.

Chemical control: Soil fumigation (methyl bromide – to be banned), seed treatment, soil drench, foliar spray.

Cyproconazole, iprodione, tolclofos-methyl, and fludioxonil are generally very effective against all major pathogenic AGs of *R. solani* and binucleate *Rhizoctonia.*

Pencycuron is highly effective against AGs 1 (rice sheath blight), 2-1, 2-2, 3 (potato black scurf), 6 and 9. It is highly effective against some AG 4 isolates but completely ineffective against others, and against AGs 5, 7, 8, *R. zeae, R. oryzae* and various binuclate *Rhizoctonia.*

These issues as well as examples of management of several crops were discussed.

The following approaches to management of *Rhizoctonia* were also dealt with in greater detail by several speakers at this workshop: crop rotation, solarization, suppressive composts, organic amendments, rhizosphere antagonistic bacteria, fluorescent *Pseudomonas* spp., *Trichoderma, Gliocladium,* induced resistance, and transgenic plants with resistance against *Rhizoctonia.*

Management of Soilborne Pathogens of Strawberry

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Strawberries are produced in California by a highly integrated system that relies heavily on sanitation and soil fumigation to manage soilborne pathogens. While the current system of strawberry production in California is very productive, the pending ban on methyl bromide (MB) necessitates further research on other approaches to managing soilborne pathogens in strawberry. Fumigation with mixtures of MB and chloropicrin (or some of the chemical options to MB) effectively controls *Verticillium dahliae, Phytophthora* species, and most other major pathogens of known importance in soil. However, soil fumigation approximately doubles strawberry yields even when known pathogens are not present, and we are currently testing the effects of rhizosphere and root-associated microbes from fumigated and nonfumigated soils on the growth and root health of strawberries.

Current California strawberry varieties are highly susceptible to Verticillium wilt and efforts are being made to find genetic tolerance. Crop rotation and/or the removal of strawberry crop debris may be helpful in future Verticillium management. Soil solarization is not practiced mainly because of the foggy coastal climate. Phytophthora root and crown rots are constant risks in strawberry production. Genetic tolerance is not sufficient and cultural methods are used widely to reduce the risk of Phytophthora root and crown rot. Cultural controls include, insofar as possible, the use of pathogen-free planting material. Soil water management is very important, with site selection and bed design directed at obtaining adequate soil drainage, and drip irrigation placement and schedules designed to minimize saturation at plant crowns. In addition, where the perceived risk of Phytophthora root and crown rot is high, Ridomil (metalaxyl) is sometimes applied through the drip system.

Management of *Pythium* Root Rot under Plasticulture and Irrigated Conditions in Arid Environments

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The genus *Pythium* contains *ca* 100 species. Major plant pathogenic species in arid environments are: *Pythium aphanidermatum, P. deliense* and *P. myriotylum.* The *in vitro* optimum temperature for vegetative growth of all of these species is 37°C. Management of root diseases caused by these high temperature species, which are associated primarily with root rot of mature plants, resides in knowledge of (i) the environmental factor(s) which favor disease onset and severity, and (ii) the relationship between soil population densities of the pathogen and disease severity. Three case studies involving *P. aphanidermatum* on various hosts (*i.e.*, sugarbeet, pepper, and spinach) were presented. Each case illustrates the critical importance of soil temperature on disease onset and severity. Specifically, soil temperatures of >27°C for 12 consecutive h or more per day at the 10-cm soil depth have been identified as the major environmental factor governing disease onset and severity. Further, fields containing pathogen population densities of 7.4 oospores/g of soil or greater, if accompanied by conducive soil temperatures, were shown to be at risk of significant disease loss. Thus, disease management strategies include preplant assessment of soil populations of the pathogen and avoidance of planting in high-disease-risk fields, selection of high-disease-risk fields for early planting and early harvest dates, and various other cultural practices which decrease soil temperatures. The last mentioned include row orientation and manipulation of the duration and frequency of irrigation.

C: MANAGEMENT OF THE BIOTIC ENVIRONMENT OF PATHOGENS

Role of Composts in Disease Suppression for Container-Grown Plants

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Several types of composts are used effectively for consistent control of diseases caused by soilborne plant pathogens in field as well as container agriculture. However, for composts to provide consistent effects many factors must be controlled. The raw materials from which composts are produced, the composting process itself, the degree to which the organic matter in composts has been stabilized, the types of microorganisms colonizing composts after peak heating, and chemical as well as physical properties of composts, all contribute to efficacy. Control of soilborne plant pathogens such as *Pythium* and *Phytophthora* spp. is due to the activity of a broad spectrum of biocontrol agents present in composts. Highly saline composts do not induce suppression unless they are applied several months ahead of planting to allow for leaching of salts. Most composts and compost-amended substrates suppress these diseases effectively. In contrast, a pathogen such as *Rhizoctonia solani*, which is controlled effectively by a narrow diversity of biocontrol agents in composts, is not controlled readily in compost-amended field soils or container media. Inoculation of composts after peak heating but before substantial recolonization with mesophiles has occurred, with inoculants of specific biocontrol agents of *R. solani*, overcomes this deficiency in natural composts. The length of time that suppression induced by composts is sustained depends on the microbial carrying capacity of the substrate. This can be characterized by the rate of hydrolysis of fluorescein diacetate (FDA), by NMR or DR-FTIR spectroscopy. Practical guidelines for FDA activity are available for container and field agriculture. Diseases of plant foliage also may be affected because some composts induce systemic resistance in plants. However, this property of composts is highly variable in nature.

The Use of Suppressive Compost for Biocontrol of Soilborne Plant Pathogens

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Composts produced from various agricultural wastes, such as composted grape marc (CGM) and composted separated cattle manure (CSM), as well as composted municipal solid waste (MSW), have been studied as to their suitability to suppress soilborne diseases. Suppressive compost can be defined as providing an environment in which disease development is reduced, even when the pathogen is introduced in the presence of a susceptible plant. Plant disease suppression is a direct result of the activity of antagonistic microorganisms, which naturally recolonize compost during the cooling phase of the composting process. Thus, compost sterilization usually negates suppression. Although the mechanism of suppression is biological in all cases, different specific mechanisms were observed in relation to various plant diseases. The mechanisms involved in suppression of *Pythium aphanidermatum*, causing damping-off in cucumbers, are of a general nature. Competition for nutrients was found to be responsible for suppression and to be affected by carbon source concentration and microbial activity. Media amended with CGM, CSM or composted MSW were also effective in suppressing diseases caused by *Sclerotium rolfsii* and *Rhizoctonia solani*. The mechanism of suppression in this case is specific, since mycoparasitism was observed. Wilt in melons and cotton as well as crown rot in tomatoes caused by *Fusarium oxysporum* were also suppressed effectively by composts. The suppression of *Fusarium* is of great importance, since fungicides are not available for this purpose and the use of methyl bromide is restricted. The employment of suppressive compost provides effective biological control of plant pathogens. The use of mature compost is essential for this purpose, as well as for healthy plant development in container media. It is therefore crucial to define parameters for determining compost maturity and use quality control measures for the application of compost in agriculture and horticulture.

Combining Organic Amendments (Bio-Fumigation) with Plastic Mulches for Soilborne Pest Management

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Chemical soil disinfestation often leads to eradication of most microbial residents in the soil. This creates a microbial vacuum which frequently leads to a rebound of pathogens that may cause even more damage than those originally targeted for control. Soils, especially those with a low microbial population, are more vulnerable to re-invasion of pathogens following fumigation. Combining organic amendments with soil solarization is a non-chemical approach to improve control of soilborne pests. Heating of soils, covered with plastic film and amended with appropriate organic material, actuates a chain reaction of chemical and microbial degradation leading to the generation of toxic compounds in the vapor and liquid soil phases. Generation of toxic compounds increases with temperature. These toxic compounds accumulate under the plastic mulch, and enhance biocidal activity against soil flora and fauna, especially soilborne plant pathogens. The plastic mulch traps the volatile compounds and creates an atmosphere in the soil which enhances the degradation of the organic matter. The toxicity of the generated volatile compounds is expected to be higher under the high soil temperatures prevailing during solarization. At the end of the process, the soil contains less pathogens, and a different microflora, which may suppress the establishment of new pathogens in this soil.

Significant qualitative and quantitative differences in volatile compounds from heated and non-heated soil amended with plant residues (crucifers, and aromatic herb plants) or high-nitrogen organic material (poultry manure, soy meal) were evident in both controlled-environment studies and field experiments. Concentrations of volatile compounds were higher in heated soil amended with plant residues than in the corresponding non-heated soil, and were directly related to increased soil heating. Numbers of propagules of *Fusarium oxysporum* f.sp. *basilici, Sclerotium rolfsii* and *Pythium ultimum* were reduced by more than 95%, when the propagules were exposed to volatile compounds from heated, amended soil. In contrast, volatile compounds from non-heated, amended soil had only a mildly lethal effect on these fungal propagules after 4 weeks of incubation. Microbial activity in heated or solarized soil amended with plant residues increased after the first 2 weeks of incubation or solarization, as compared with non-amended soil. Apparently, microbial activity is involved in pathogen control during solarization of amended soil.

The effect of solarization of organic amended soil as a control method was validated under commercial field conditions. Viability of fungal propagules was completely eliminated to a depth of 50 cm in field experiments in solarized amended soil. Crown rot of tomatoes, Verticillium wilt of potatoes, and root-knot nematodes in lettuce were controlled effectively by combining soil solarization with poultry manure or soymeal amendment. Long-term effect of solarization treatment was evident on Verticillium wilt of potato and on root-knot nematodes in lettuce for at least two crops after the solarization treatment, lending further support to the involvement of biological processes.

New Perspectives in Biocontrol of Plant-Parasitic Nematodes

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Comprehensive studies in our laboratories revealed the nematicidal potential of waste products from crustacean shells and other proteinaceous compounds for controlling *Heterodera* spp., *Meloidogyne* spp. and *Tylenchulus semipenetrans.* Control of *M. javanica* was compared in irradiated and non-irradiated soil to which the waste product had been added and an active role by microorganisms was indicated. *Telluria chitinolytica* sp. nov. and *Bacillus cereus*, new bacteria which were isolated, grown and identified in our laboratory, appeared to have potential as bioagents for controlling phytonematodes. Intensive studies of the mode of action of these bacteria were carried out and the use of *T. chitinolytica* for phytonematode control was patented. *Trichoderma* isolates were tested in pots (*T. harzianum* [T-203] and *T. lignorum*), in 50-*l* containers (*T. harzianum*) and in 1000-*l* microplots (*T. harzianum*) for their nematicidal activity against the root-knot nematode *M. javanica.* Both isolates revealed an increase in top fresh weight and yield, and a decrease of galling indices, as compared with nematode-infected non-treated plants. Preliminary characterization of the possible active component in the T-203 isolate filtrate showed it to be a nonvolatile, moderate (6–8) pH, water-soluble and heat-stable compound with molecular weight < 3000 kDa. A fungal parasite was isolated from black-colored egg masses of *M. javanica* on tomato roots. The fungus did not sporulate on any of the culture media tested or in the egg mass. Hyphal characteristics suggest that it is similar to the hyphomycete genus *Scytalidium.* Hyphae of the *Scytalidium*-like fungus (CBS 645.97 and IMI 368886) proliferated in the gelatinous matrix of the egg mass and penetrated the eggshell *via* a penetration peg. Parasitism of the egg mass greatly lowered hatch rate of *M. javanica* juveniles *in vitro.* The nematode population in soil treated with the fungus was lower than in non-treated soil after one nematode generation. The exact identification of the *Scytalidium*-like fungus, the technology for mass production, and its application in the field for control of root-knot nematodes, require further investigation. The specific adhesion of microorganisms to the cuticle of phytonematodes occurs on the surface coat (SC). We have started to investigate the biological role of the SC, hoping that the information gained by research on this topic will provide a platform for the development of new approaches to enhance biological control of nematodes through a better understanding of the mechanisms of adhesion by bacterial pathogens.

Commercial Biological Control of Root Pathogens of Vegetables with Products Containing a Rhizosphere Competent Strain of *Trichoderma*

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An objective of this laboratory at Cornell and, recently, the commercial company BioWorks, has been to produce biological control products for use in commercial agriculture and horticulture. Strain 1295-22 of *Trichoderma harzianum* was produced using protoplast fusion. This strain possessed both strong abilities as a biocontrol agent against various pathogenic fungi and a high level of rhizosphere competence. Several products are sold that contain this organism. All of them result in colonization of roots, which results in more robust roots. Plants with better root systems are able to withstand various stresses, including disease, drought and suboptimal nutrition. As a consequence, yields frequently are increased in row crops. In greenhouse crops, one application of the biological usually is as effective as several applications of conventional fungicides. For transplanted vegetable crops, roots colonized by treatment in the greenhouse may result in better transplant survival, control of root diseases in the field, and increased yields. Control of foliar and fruit pathogens also is possible. Spray applications of T-22 controlled foliar phases of *Botrytis* and have the ability to control powdery mildew as well. For strawberries, T-22 can be applied to flowers either by a spray or by bees that exit hives through a T-22 preparation.

Potential for Biological Control of Root Pathogens with *Pseudomonas* Species Introduced into the Rhizosphere

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The major limitation of chemicals as seed treatments for soilborne pathogens is lack of root protection for more than 1–2 cm below the seed. We have focused on the use of rhizobacteria, particularly *Pseudomonas* species, (i) able to colonize and maintain populations in the rhizosphere of wheat 5–10 cm and more below the seed, (ii) able to produce one or more antibiotics inhibitory to the target root pathogens, and (iii) tolerant to seed-treatment chemicals, needed for immediate protection of germinating seeds. Our strains are from the rhizosphere of wheat growing in soil from fields where wheat had been grown continuously for many years, to help ensure that the strains are rhizosphere-competent on the crop intended for protection. Initially, we concentrated on *P. fluorescens* 2-79 and *P. aureofaciens* 30-84 with ability to produce phenazine (PHZ) antibiotics. We are continuing basic work on genetics and molecular biology of the phenazine mechanism as a model system, but have discontinued field-testing these strains because they are ineffective in high-clay soils. The second phase of our field work concentrated on *Bacillus* species L324-92 with antibiotic activity against three wheat root diseases, and on *P. fluorescens* Q69c-80 with no known ability to produce antibiotics, but widely effective in the field. In the current (third) phase of our work, we are also now concentrating on *P. fluorescens* Q8R1-96 with ability to produce the antibiotic 2,4-diacetylphloroglucinol (PHL). The evidence is strong that PHL-producing strains like Q8R1-96 account for take-all decline. Used as a seed treatment, this strain produced the highest yields of wheat at every location where tested in 1997. We now have cultures of Q8R1-96 transformed to produce PHZ in addition to PHL.

Induced Resistance Using Plant Growth-Promoting Rhizobacteria (PGPR)

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One of the mechanisms by which PGPR may exhibit biological disease control is through enhancement of host defense responses when the PGPR act as inducing agents to activate induced systemic resistance (ISR). Over the past 8 years, a team of researchers at Auburn University has studied the phenomenon of PGPR-mediated ISR, and the results indicate the following. Selected PGPR strains belonging to diverse Gram-positive and Gram-negative genera can, upon seed treatment or soil drench treatment to plant root systems, reduce the incidence of distally infecting pathogens. Single PGPR strains have been shown to reduce pathogen infection and symptoms of multiple diseases on cucumber and tomato. Cucumber diseases affected in both greenhouse and field studies in multiple years include foliar diseases (angular leaf spot, caused by *Pseudomonas syringae* pv. *lachrymans*; and anthracnose, caused by *Colletotrichum orbiculare*); systemic wilt diseases (cucurbit wilt, caused by *Erwinia tracheiphila*; and Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cucumerinum*), and the systemic viral disease caused by cucumber mosaic virus (CMV). In the case of cucurbit wilt, disease control is linked to PGPR-mediated reductions in plant preference by the insect vectors, the striped and spotted cucumber beetles. In field and greenhouse studies, PGPR treatments led to significant reduction in beetle feeding, which was associated with PGPR-mediated reductions in cucurbitacin C, a feeding attractant. With tomato, protection has been noted in the greenhouse or field against CMV; bacterial spot, caused by *Xanthomonas axonopodis* pv. *vesicatoria*; tomato mottle geminivirus; and bacterial speck, caused by *P. syringae* pv. *tomato*. Mode of action studies support the conclusion that the observed systemic biocontrol results from ISR, since measurable biochemical and cytological changes occur in the plant in relation to host recognition of the inducing PGPR strains. The specific plant changes vary somewhat among PGPR strains and are the focus of intense current investigation. To date, we have observed enhanced peroxidase activity and lignification in cucumber and induction of PR1a promoter in transgenic tobacco containing a GUS reporter gene. In this tobacco system, all PGPR strains which enhanced protection against wildfire disease – caused by *P. syringae* pv. *tabaci* – in the greenhouse induced GUS activity, whereas control strains lacking disease protecting activity did not induce GUS activity significantly relative to controls.

Resistance to Soilborne Diseases in Tomatoes: A Breeder's Point of View

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In recent decades breeders have provided a significant remedy for the damage caused by soilborne pathogens to open-field and greenhouse tomatoes. Introductions of wild *Lycopersicon* spp. from the center of origin, genetic studies, and introgression of desired genes into the domesticated tomato have provided breeders with tools for a good solution to epidemics of Fusarium and Verticillium wilts, nematodes, Fusarium crown root rot, bacterial canker, corky root and others. Based on a breeder's experience, we shall hereby deal with only a few case histories, with the aim of strengthening the understanding and ties between breeders and plant pathologists.

The domesticated tomato does not contain genes for resistance to pests, and therefore when a new pest or pathorace appears, breeders have to turn to the wild *Lycopersicon* genepool. The first time resistance was introduced into tomatoes was in the early 1940s, in Missouri, where the gene *I* was introgressed from *L. pimpinellifolium*, thus providing resistance to *Fusarium oxysporum* f.sp. *lycopersici*. This event did not go smoothly, as the back-crossed population segregated abnormally and showed an unexpected preference for resistant plants. Moreover, the dominant gene *I* exhibited incomplete penetrance, thus resulting in some wilting of infected heterozygous plants. Thereafter, newly bred resistant cultivars became popular all over the USA and Europe. In Israel, the gene *I* was introduced by N. Kedar from cv. 'Homestead' into the locally grown French cv. 'Marmande' and early in the 1960s the Israeli tomato cv. 'Rehovot 13' was released. Soilborne pathogens are supposed to spread slowly and consequently the appearance of new physiologic races is also slow. Yet in the early 1960s, a new physiologic race spread rapidly throughout Florida, causing heavy losses to the local tomato industry. Here, again, the wild genepool was utilized to provide a fairly quick solution to the problem. Learning from experience, we in Israel introduced material with the I_2 gene, made the first necessary crosses and started a national survey to detect the new race. The moment the first report came in, breeders started to act and in a rather short time released new resistant cultivars and thus prevented much of the potential damage. Since no information was available as to whether or not I_2 provided protection against race 1, breeders all over the world have incorporated the two genes into most tomato cultivars. Back in the 1960s Verticillium wilt (caused by *Verticillium albo-atrum* and *V. dahliae*) also became a threat, and the cooperation among plant hunters, geneticists and breeders proved successful once again, resulting in new releases resistant to this soilborne pathogen. Recently, reports from Australia and Florida indicate the appearance of a third race of *Fusarium*. A new pathorace of *Verticillium* has also been reported in Morocco, Canada and elsewhere. Geneticists have once again located genes in the wild genepool, which provide resistance to these new pests. However, with the intensification of farming and extension of the growing seasons, it became obvious that modern cultivars should incorporate both resistances (VF plants), and that nematodes not only injure plants but also facilitate the infection of VF plants with Fusarium. Hence, the introduction of the gene *Mi*, from *L. peruvianum*, became essential. However, plants homozygous for the *Mi* gene suffer from low fertility. This gene, therefore, could be used only in the heterozygous state, and breeding of parental lines with good combining ability became essential. The incorporation of an increasing number of genes for resistance has become a routine component of breeders' activity. Not only horticultural traits have to be dealt with, but also a battery of genes which allow for safe cultivation of the tomato hybrids. The large number of desired resistances led breeders to suggest grafting of genotypes with excellent horticultural traits on rootstocks with combined resistances to soilborne pests. In Europe and Israel this technology, which provides means for efficient disease management, is still labor-intensive, whereas in Japan and Korea, where grafting of vegetables is common, engineers have perfected robotics for mechanical grafting, thus making the use of grafted plants possible on a very large scale.

Potential for Use of Transgenes to Manage *Rhizoctonia* Root Rot

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The mycoparasite *Trichoderma harzianum* is a fungus present in almost every soil, and is antagonistic to other fungi. As a result of recognition and contact between *Trichoderma* and a target hypha, a group of extracellular lytic enzymes is produced, including β-(1-3)-glucanases, proteases and chitinases. Chitin is a major structural cell wall component of plant pathogenic fungi. The polymer is degraded by chitinase, an enzyme which plays a substantial role in biological control. We have introduced the cloned chitinase from *Serratia marcescens* (*chiA*) in a vector into *Escherichia coli.* In greenhouse experiments, significant suppression of disease caused by *Sclerotium rolfsii* was detected when bean seedlings were irrigated with the engineered *E. coli.* Similarly, this gene was introduced into *Rhizobium meliloti* which became antagonistic to pathogenic fungi. Our *chiA* was also introduced into tobacco plants and significantly increased their resistance to *Rhizoctonia solani.* We have cloned the same gene into the *Trichoderma* genome under the 35S constitutive promoter from CaMV. The transformed *Trichoderma* secreted high levels of chitinase with no induction by chitin. Our results have shown that in dual culture, the transformants have a remarkable advantage in lysing *S. rolfsii* compared with the wild type. The chitinolytic system of *Trichoderma* spp. has not yet been fully resolved. We have employed a set of three fluorescent substrates to identify chitinolytic activities of proteins excreted by *Trichoderma.* The system was found to be composed of six distinct enzymes (one of which has not been reported previously): two β-1,4-N-acetyl-glucosaminidases (CHIT102 and CHIT73) and four endochitinases; the newly described enzyme is CHIT52. All chitinolytic enzymes were induced and excreted only when *Trichoderma* was grown on chitin as the sole carbon source. Differential expression of some of the enzymes was found when attacking different pathogenic fungi. Application of some microbial genes encoding for active antifungal proteins can serve both for improving biological control as well as to induce resistance in plants.

Breeding Peas for Resistance to Root Diseases

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Peas are susceptible to several soilborne fungal pathogens. *Pythium* spp. cause seed root, damping-off and root tip pruning. There are three types of resistance to *Pythium*: (i) seeds lose susceptibility within 48 h after imbibition; (ii) round seeds exude less substances stimulatory to *Pythium*; and (iii) pigmented seed coats are fungistatic due to phenolic compounds. Rhizoctonia seed, seedling, hypocotyl and/or epicotyl rot is caused by the anastomosis group AG-4 type. Only peas with vigorous, thick stems, which emerge rapidly, escape serious damage. Common root rot, caused by *Aphanomyces euteiches*, is the most important root disease worldwide. Tolerance to common root rot was reported associated with three undesirable, dominant alleles at three unlinked loci. Substitution of recessive, desirable alleles reduced tolerance. However, resistance to common root rot has been developed in breeding lines with acceptable horticultural traits. Resistance is evidenced by slower pathogen multiplication in roots and slower lesion spread on resistant roots. The additive, resistant gene effects are being studied *via* recombinant inbred lines (RIL's) of crosses between resistant and susceptible parents. Quantitative Trait Loci markers are being developed using these RIL's and were discussed. Fusarium root rot caused by *F. solani* f.sp. *pisi* can increase pea yield losses from a constant 10% to at least 50%. Unlike common root rot, Fusarium root rot is reduced by cultural practices. Because *F. solani* is a relatively slow root invader and colonizer, restriction of rapid root growth favors *Fusarium*. In addition, good seedling vigor is a must in any procedure to evaluate pea lines for resistance to *Fusarium*. Electroconductivity measurements are conducted on individual seeds prior to soaking in conidial inoculum. The genetics of increased rooting vigor is being studied with the aim of combining genetic resistance to Fusarium and common root rot with increased rooting vigor.

E: IMPLEMENTATION OF INTEGRATED MANAGEMENT OF DISEASES CAUSED BY SOILBORNE PLANT PATHOGENS AND PRIORITIZATION OF FUTURE RESEARCH NEEDS

Control of Soilborne Pathogens by Inducing Soil Anaerobiosis

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The method proposed here is aimed at controlling soilborne pathogens by imposing a general soil anaerobiosis. This strategy was suggested previously by others but to the best of our knowledge has never been tested under field conditions. The method involves the incorporation of green plant material (either broccoli or grass, ~40 t fresh weight/ha), followed by irrigation and application of a plastic tarp with a low permeability for oxygen, for 12–15 weeks. The method was tested in field experiments performed in four consecutive years.

Following application of the plastic tarp, the soil became anaerobic rapidly, irrespective of the incorporation of plant material. In tarped soil with plant material the redox potential (Eh) dropped gradually to values as low as −200 mV at a depth of 15 cm, indicating strongly reducing soil conditions. In tarped soil without plant material, the redox potential did not reach these low levels. Soil temperatures did not reach (sub)lethal levels in these experiments. The effect on survival of pathogens was tested by burying nylon bags with ~100 ml inoculum at various depths and determining the viability after the treatment. In tarped soil with plant material, survival of *F. oxysporum* f.sp. *asparagi, Rhizoctonia solani* and *Verticillium dahliae* was greatly reduced in all experiments (usually by 90–100%). In treatments with plant material or plastic only, no significant reduction in pathogen survival was found.

Pathogen inactivation was similar for broccoli and grass. Pathogen inactivation is suggested to be caused by a direct effect of low oxygen levels in the soil atmosphere, or by products of fermentative bacteria that accumulate temporarily in the anaerobic soil prior to the onset of methane production, or by a combination of the two factors.

Molecular Approaches to Enhance Biocontrol of Phytopathogens

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Several isolates of soilborne, rhizospheric bacteria, mainly of the *Enterobacter* and *Pseudomonas* spp., were shown to be able to suppress growth of approximately 30 species of fungal and bacterial phytopathogens *in vitro*. The results of experiments in greenhouse have shown that the strains could be considered as new, potential biological control agents. The application of the bacteria reduced the incidence of root-rot disease caused by *Rhizoctonia solani* in cotton and beans, and of damping-off caused by *Pythium aphanidermatum* in cucumber up to 90%. The high potential of the isolates was shown also in model experiments with *Sclerotium rolfsii* in beans and *Fusarium oxysporum* in melon. Some of these strains possess a unique combination of multiple mechanisms, which provided their action as strong antagonists of phytopathogenic microflora. Thus, an isolate of *Enterobacter agglomerans*, which showed its high efficiency under greenhouse conditions, is able to produce and excrete into the culture medium a complex of chitinolytic enzymes, which included two exochitinases and one endochitinase. The structural part of the gene *chiA* encoding the endochitinase was cloned in *Escherichia coli* under control of its own promoter or *lac* promoter. The expression of the cloned chitinase in the hybrid *E. coli* strains provides them with the ability to excrete the endochitinase and to protect cotton against *R. solani*. In addition, the *E. agglomerans* strain produces the antibiotic pyrrolnitrin, which has a wide range of antifungal activity. The combination of chitinases with this antibiotic, which has a direct effect on membranes, could be considered an advantage, *i.e.*, as an aid to the chitinases in their attack on fungal phytopathogens. Further enhancement of some of these bacterial antagonists' biocontrol potency can be achieved by extending the assortment of antifungal compounds produced by the bacteria and by genetic manipulations with systems which regulate production of these compounds on a global level of individual genes expression.

Biofungicides: The Requirements of the End User

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A number of microorganisms have been used for many years for the biological control of soil as well as foliar fungal diseases. Despite the proven efficacy of many of the biological products developed, their market share in the overall fungicide market has remained very small. There are several reasons that could explain why these products are not yet used extensively by the farmers. Consistency of results, cost and stability of the final product have been the major difficulties encountered with the many biofungicides introduced onto the market. Biofungicides will not be used extensively until proper solutions are offered for these problems. The present attitude in most of the developed countries is very favorable to the introduction of environment-friendly pesticides. Legislation around the world is promoting the use of biorational products, but it is the satisfaction of the end user that will make the difference between success and failure.

The establishment of strict quality control methods could solve the problems related to consistency of results. This often involves the development of new bioassays as well as of techniques to study the physico-chemical parameters of the final formulation. The definition of environments and crops in which the product can be used is also important. It should be kept in mind that the active ingredients of biofungicides can function only within certain limits of temperature and humidity, parameters that vary with the geographical location in which the product is to be applied. The different types of soil in which these materials are applied also play an important role in determining their efficacy and the consistency of results.

Mass production of microorganisms using efficient and cost-effective fermentation techniques should be able to provide products that can be offered at competitive prices. The use of cheap and readily available raw materials and the increase in fermentation yields in shorter production cycles are the main objectives to be achieved. The liquid fermentation of fungi as the active ingredients of biofungicides is a longer and more difficult process than the one needed for the manufacture of biofungicides based on bacteria or yeast. In many cases the production of these active ingredients by solid fermentation should be considered.

As with many other biopesticides, formulation remains one of the most important aspects in need of further development. The availability of a proper formulation will influence all the parameters involved in manufacturing a viable product. A good formulation will not only increase the stability and shelf-life of the product, but will also have an impact on the price as well as on the consistency of the results obtained in the field. Finding the right formulation for biofungicides is not an easy task, mainly due to the limitation of materials that can be used for this purpose and to the specific requirements for survival and growth of the different microorganisms used as active ingredients. In the last few years significant progress has been made, but there is still room for improvement.

As solutions to the difficulties indicated above become available, the share of biofungicides in the market will increase significantly. The introduction of biofungicides as part of Integrated Pest Management is the main objective of all the companies involved in the Research and Development of these products.

What is a Beneficial Rhizosphere Microorganism?

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Beneficial rhizobacteria have been termed plant growth-promoting rhizobacteria (PGPR). Mechanisms involved in growth-promotion by PGPR can be classified as indirect or direct effects. Indirect mechanisms generally involve aspects of biological control, whereas direct effects include nitrogen fixation, production of hormones, and enhancement of nutrient uptake. Relatively little is known about mechanisms associated with direct growth-promotion, probably because few acceptable model systems exist for studying root/microorganism interactions. We have developed a gnotobiotic bioassay, using potato plantlets derived from single-node explants grown in tubes containing solidified agar medium. Studies with this model system serve to illustrate some important features that may be expected from PGPR/plant interactions. Growth-promotion of potato by PsJN (*Pseudomonas* sp. [strain PsJN]) was cultivar-specific. Inoculated plantlets of cv. 'Norchip' showed a five- to eightfold increase in root weight, cv. 'Kennebec' a two- to threefold increase, cv. 'Shepody' no response, and cv. 'Chaleur' a decrease of 50%. PsJN was shown by other researchers to promote growth in a number of crop species, but to be cultivar-specific with them also. In laboratory studies PsJN consistently promoted growth of potato, but in the field the response was site-specific. Bacterized plants had improved growth and yield of tubers in Alliston soils, but were reduced in Simcoe soil. This effect was reproduced under growth room conditions. Pasteurization of Simcoe soil eliminated the inhibitory impact of PsJN, suggesting that the effect was determined due to presence of soil organisms. Therefore we investigated the interactions of PsJN with other bacteria in the geocaulosphere. We harvested tubers at 2-day intervals from each soil, sterilized their surface, made a liquid homogenate, and plated the homogenates onto nutrient agar. A dozen bacteria isolates were recovered and identified by fatty acid profiles and biochemical tests. All genera isolated were found in tubers from both soils except *Pseudomonas acidovorans*, which was present only in tubers from Simcoe soil. The presence of PsJN significantly altered the types and populations of bacteria recovered from the tubers grown in non-pasteurized soil. However, four isolates, *Serratia proteamaculans, P. acidovorans, Alcaligenes piechaudii* and *Rahnella aquatilis*, showed antagonism to PsJN in assays on agar media. Several isolates also inhibited growth of potato nodal explants. Combinations of PsJN with each of the bacteria isolates showed that *Pantoea agglomerans*, which by itself had neutral or beneficial effects, when combined with PsJN was inhibitory to the growth of plantlets.

Use of *Trichoderma* spp. in the Management of Fusarium Root Rot of Beans

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Diseases, pests and soil fertility are major limiting factors in bean production in Kenya, where small-scale farming is practiced. Mean yields are between 500 and 600 kg/ha, whereas the potential is over 2,000 kg/ha. Root and stem rots are among the most economically important diseases and reduce yields significantly. The western part of the Kenyan highlands is hit badly by bean root rots and every effort is being made to alleviate the problem. In this study, two *Trichoderma* isolates, coded T3A and T13, preserved at the Department of Crop Protection (University of Nairobi), were screened for antagonism *in vitro* against *Fusarium oxysporum* f.sp. *phaseoli*. The pathogen had been isolated from diseased beans from Western Kenya and had proved pathogenic. Isolates T3A and T13 suppressed colony growth of *F. oxysporum* in potato dextrose agar by 92% and 56.4%, respectively. The suitability of three materials: autoclaved half-broken dehulled rice, sorghum and vermiculite, as carriers of the antagonistic *Trichoderma*, was tested. Broken rice produced the best growth as determined by spore count after 14 days, followed by sorghum, whereas vermiculite was the poorest carrier. The two *Trichoderma* isolates also varied in their growth, with T3A outdoing T13. The antagonist and the type of carrier used determined the level of disease management in the greenhouse. The best control, as determined by disease incidence in the 7th week, was achieved by T3A in broken rice (32% disease), followed by T13 in sorghum (61%), T3A (73%) and T13 in vermiculite (74%), compared with 75% in the control. There was some correlation between saprophytic growth of the *Trichoderma* isolates in different carriers in the laboratory and their ability to suppress bean root rot in the greenhouse. In future studies, isolates of *Trichoderma* on the good carriers and other agricultural organic wastes as carriers should be tested under field conditions for bean root rot management.

Advances in the Biological Control of *Verticillium dahliae*

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Successful biological control of *Verticillium dahliae* could be achieved, *inter alia*, through: (i) Inhibition of microsclerotia formation on the diseased plant tissues or reduction of the survival of formed microsclerotia in plant tissues by fungal or bacterial antagonists during host disintegration; (ii) fungal antagonists affecting the survival of microsclerotia weakened by sublethal solarization or sublethal fumigation; and (iii) fungal or bacterial antagonists preventing root infection of germinating microsclerotia. Preliminary data obtained from *Verticillium*-infected cotton or other solanaceous hosts showed that the fungal bioantagonist *Talaromyces flavus* is not able to prevent microsclerotia formation but can affect survival or vigor of already formed microsclerotia. Recent data have shown also that *T. flavus* is able to be established preferentially in the zone of elongation. This constitutes a useful biocontrol property since it enables the antagonist to interfere with the entrance of the pathogen into the root tips through various mechanisms and also further to reduce pathogenicity of weakened propagules.

Rhizosphere or endorhizosphere bacteria with preferential ability to occupy the root tips and to exercise antifungal activity against *V. dahliae* are of primary importance. Selective media such as 523 were used to isolate mainly endorhizosphere *Bacillus*. Selection and evaluation of rhizosphere bacteria as biocontrol agents against *V. dahliae* showed the occurrence of potential antagonistic rhizosphere bacteria in the interior of the root tips (endorhizosphere). Nearly 500 bacterial isolates were isolated from tomato root tips grown in solarized soils. *In vitro* evaluation of their ability to form antagonistic zones against *V. dahliae* demonstrated that 12% produced antibiotics in dual cultures against *V. dahliae*. Finally, 18 *Bacillus* isolates (17 antibiotic-producing and one not-antibiotic-producing) were investigated in glass-house or field evaluation. *In planta* glass-house evaluation performed with eggplants, treated at the one-leaf stage by root dipping in a 10^8 cfu/ml bacterial suspension of each selected isolate, with a second application following 20 days later before transplanting to soil infested with 50 microsclerotia/g soil, demonstrated that two endorhizosphere bacterial isolates, designated K-165 and 5-127, were the most efficient in reducing symptom development; there was 40–70% reduction compared with the untreated controls 2 months after transplanting. These isolates, further evaluated under field conditions as seed dusting, delayed or reduced symptom expression caused by *Verticillium* wilt and increased yield by 25% in field-grown potatoes.

Regarding the ecology of the tested *Bacillus* isolates, it was shown that they were able to colonize the rhizosphere of various solanaceous hosts. Application of a bacterial culture suspension of K-165 through the irrigation system of tomatoes grown under plastic-house conditions showed that the rhizoplane and the vascular tissue harbored the antagonist more than 2 months after the application at figures over 10^4 cfu/g fresh weight of roots. Similarly, investigation for endophytic presence of the antagonist demonstrated its existence in the vascular tissue at figures exceeding 10^4 cfu/cm^3 of vascular tissue. Both antagonists could also occupy the rhizosphere and endorhizosphere of eggplants grown under glasshouse conditions, as was shown by using rifR mutants of the isolates at various stages of plant growth.

As for the mechanisms of action of these two endophytes, beyond their antibiotic-producing ability and chitinolytic activity, their ability to produce indole-butyric acid was demonstrated. Furthermore, the possibility of induced resistance can not be excluded. Improvements in the mode of application of the *Verticillium* biocontrol agents by using pellets for fungal antagonists and dusts for the bacterial ones, could result in a more efficient biocontrol of *Verticillium* wilt of potatoes, or by seed coating for *Verticillium* wilt of cotton. Preliminary approaches to control *Verticillium* wilt of olive trees in Greece involve the use of a bacterial suspension of the antagonist injected into the soil around the root system of diseased trees. The antagonist is mixed before injection with inert granules possessing a high water-absorbing capacity (Water Works ^{TM}) and a liquid fertilizer. Bacterial suspensions of biocontrol agents were also applied with good results through the fertilization equipment in tomato plastic houses.

Strategies for Management of Root Diseases in Direct-Seeded, Cereal-Based Cropping Systems

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Direct-seeding ('no-till farming') is replacing conventional farming for agronomic crops because it permits more intensive cropping, higher yields, and lower operating costs while saving or rebuilding soils. Take-all, Rhizoctonia root rot and Pythium root rot each are favored by direct-seeded cropping systems that depend on wheat and/or barley more that 50% of the time, typical of the U.S. Northwest. Our work shows that yields approaching those in fumigated soil (used as a standard) can be achieved when all practices outlined below are followed. (i) Eliminate volunteer wheat and barley plants and grass weed hosts, in order to have a plant-free period for at least 2 weeks before planting the next crop, and thereby minimize carryover inoculum, especially of *R. solani.* (ii) Plant new, high-quality seed, for maximum tolerance to *Pythium.* (iii) Clear crop reside from within the seed rows, placing it between the rows, to favor warming and drying of the top few centimeters of soil around the plants, where these pathogens are most active. (iv) Plant the rows in pairs, rather than spaced uniformly, to keep the crop canopy open longer into the growing season and favor more warming and drying of the top layer of soil. (v) Plant at an angle to the old stubble rows, in order to place maximum amount of seed between, rather than directly into, the old stubble rows where the inoculum potential of root pathogens is highest. (vi) Place fertilizer directly below the seed, within easy access of roots for when they become infected. (vii) Treat the seed with fungicides active against the root pathogens. Work is now well advanced on the combination of chemical seed treatments with antibiotic-producing rhizobacteria active against all three root diseases.

Management of Soilborne Pathogens of Protected Crops: Results in Italy

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Soilborne pathogens pose a serious threat to most protected vegetable and ornamental crops. The recent restrictions in the use of methyl bromide, a fumigant widely applied in Italy (the second largest consumer in the world after the USA), strongly increased the interest into the search for alternative control measures, effective technically and also reliable from an economic point of view. The research carried out over the past few years in order to develop biocontrol agents effective against Fusarium wilts and to employ them in integrated control strategies, was summarized. Antagonistic *Fusarium*, isolated from *Fusarium*-suppressive soils, have been developed and widely exploited on a number of crops. Their use in integration with physical (soil solarization or steam) or chemical control measures resulted in very satisfactory wilt control. Soil solarization, carried out alone for 4 weeks or combined with other measures for shorter periods, proved effective against *Rhizoctonia solani, Pythium* spp. and *Sclerotinia sclerotiorum.* Carnation, cyclamen and basil were chosen as model crops. The results obtained by integrating the use of genetic, biological, physical and chemical control measures were presented and critically discussed.

Integrated Control of Damping-off of Rooibos Tea (*Aspalathus linearis*) Seedlings in Nurseries

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Damping-off of rooibos tea [*Aspalathus linearis* (Burm. f.) R. Dahlg.] seedlings in nurseries in the Western Cape Province of South Africa leads to serious losses. The disease is caused by a complex of fungi of which *Fusarium oxysporum, F. solani, Pythium irregulare* and *Rhizoctonia solani* are the most important. Studies of the role of seed, soil and in irrigation water as sources of inoculum of pathogenic fungi showed that the two latter are the major sources. Attempts to reduce inoculum in soil and irrigation water included soil solarization and treatment of soil and irrigation water with a quaternary ammonium compound. Seed and soil treatments with fungicides were also investigated as part of an integrated control strategy against damping-off. Contradictory results were obtained with soil solarization (conducted 1 month before planting). In the low rainfall area (180–250 mm/year), soil solarization significantly decreased survival of seedlings, whereas it significantly increased survival in the high rainfall area (> 250–400 mm/year). The survival of seedlings subjected to irrigation water/soil drench treatment combinations which included the quaternary ammonium compound was considerably higher, and the incidence of *F. oxysporum* and *Pythium* spp. on seedlings lower, than treatment combinations without this compound. Seed treatment and a soil drench with fungicides increased survival of seedlings, was not phytotoxic and was economically beneficial. The reduction of inoculum of pathogenic fungi in soil and irrigation water and the protection of seedlings against pathogenic fungi using seed and soil treatments with appropriate fungicides are important components of an integrated control strategy against damping-off of rooibos tea seedlings in nurseries.

Investigations on Root Health and Rhizosphere Ecology of Strawberry

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With the likely loss of methyl bromide availability for soil fumigation at the end of this decade, a better understanding of which pathogens are responsible for yield losses in the field will be of help in the development of effective control strategies. In addition, a more comprehensive understanding of the rhizosphere ecology would be helpful not only for determining whether deleterious rhizosphere colonizers contribute to yield reductions, but also for the identification of beneficial microflora that might be capable of improving root health. A number of pathogens of strawberry and other rotation crops have been recovered from the root system of strawberry plants. The primary focus of the research to date has been with approximately 8–10 *Pythium* spp. and three putative AGs of *Rhizoctonia fragariae*, which are currently under evaluation for virulence and host cultivar susceptibility. Field evaluations in which the pathogens are added back into the beds prior to transplanting are in progress to determine whether greenhouse results correlate with field observations. The general rhizosphere ecology of plants grown in these trials also has been examined. A range of fungi, bacteria and actinomycetes has been recovered from the roots of plants grown in fumigated and nonfumigated soils at different times during the growing season. A number of these isolates have been evaluated for their effect on plant growth. Several isolates were identified that significantly increased shoot and root growth in growth chamber tests when plants were grown under conditions similar to those encountered for winter-transplanted crops. Field trials during the last season identified isolates that appeared to have a beneficial effect on plant establishment, early season growth, and yield. More extensive field evaluations with these isolates are currently in progress.

Etiology of Apple Replant Disease and Prospects for Its Control through Integrated Management Strategies

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Due to the complex and often uncertain etiology of apple replant disease, control of the disease has been dependent upon the use of pre-plant soil fumigants. As an initial step in development of biologically based disease control alternatives, a systematic examination of the etiology of apple replant disease was conducted in Washington State. The approaches utilized in these studies include characterizing the microbial community from the roots of symptomatic and healthy trees; examining the impact of semi-selective biocides and soil pasteurization on plant growth; monitoring changes in rhizosphere microbial communities in response to these soil treatments; and determining the pathogenicity of microorganisms commonly isolated from symptomatic root tissues. It was demonstrated that a fungal complex composed of species belonging to the genera *Cylindrocarpon, Phytophthora, Pythium* and *Rhizoctonia* is the dominant cause of apple replant disease in Washington. Surprisingly, composition of the pathogen complex was consistent across the study sites, although the relative dominance of individual components varied among orchards. In contrast to previous studies conducted in other regions, the lesion nematode, *Pratylenchus penetrans*, was shown to have a limited or no role in disease development.

Identification of the causal pathogen complex enabled the formulation of alternatives that will utilize biological, cultural and narrow-spectrum chemical controls in an integrated system for the management of apple replant disease. In conjunction with efficacy trials, the impact of control measures on the resident soil microflora has been documented. These studies were prompted by results from preliminary trials in which certain pathogen-specific control measures exacerbated disease symptoms, and the findings have been instrumental in defining the cause of disease control successes and failures. In turn, this information has been used to modify disease control protocols. These studies have also provided insight into practices which may enhance the activity of specific disease-suppressive elements of the resident soil microflora.

Integrated Management of Peanut Pod Diseases: Pythium-Centered Pod Rot and Delimited Shell Spots

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Soil moisture is conducive to both *Pythium* activity and fruit set of peanuts (groundnuts). Therefore, after the peak of flowering, studies were conducted of infrequent irrigations, which caused the topsoil to dry out between irrigations, whereas the continuous moisture of the subsoil sustained the plants. Since the finding that this procedure is successful, elevated seedbeds, which facilitate the draining of the geocarposphere, have been used in Israel. Subsequently the combination of metham-treated soil and elevated seedbeds was found to be successful and became a frequent practice. A susceptible commercial peanut cultivar ('Shulamit') was then compared experimentally with a semi-resistant breeder line (FA6) in metham-treated and untreated plots. In the untreated soil the incidence of rotted pods was 37% in the susceptible cv. and 19% in the breeder line. Metham treatment reduced pod-rot incidence to 12% in the susceptible cv. and to 2–3% in the breeder line; soil treatment and resistance were thus additive. Another disease, delimited shell-spots (DSS), is caused by an unidentified primary factor in slowly draining, poorly aerated soil, when legumes are included in the crop rotation. Small cereals in the rotation, cut for green fodder, alleviated the situation. Metham sodium soil treatment reduced DSS, but it was efficient only with a fairly resistant breeder line (AZ1). In another trial, with a highly susceptible breeder line, solarization was included and found to be efficient: 53% spotted pods *vs* 91% in the control. Metham sodium reduced the incidence of spotted pods to 82% only. A reduction to 22% spotted pods was attained by a combined, synergistic treatment of solarization and metham sodium.

Organic Amendments for the Control of Soilborne Plant Pathogens: An Economic Case Study for Ontario

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Organic amendments can control soilborne plant pathogens. For successful adoption, use of organic amendments must be demonstrated to be profitable. Accordingly, a field experiment was initiated on two potato farms near Alliston, Ontario. Each farm was heavily infected by *Verticillium dahliae* and *Streptomyces scabies*. In the spring of 1996, to three plots (7.6 × 3.6 m) on each farm, meat and bone meal (40 t ha^{-1}) or poultry manure (75 t ha^{-1}) was incorporated to a depth of 15 cm. Total potato yield and percent marketable yield were measured in 1996 and 1997. The profit margin for each treatment was estimated as the difference between income (marketable yield × market value) and estimated total production costs. A typical field not infected by *V. dahliae* or *S. scabies* was estimated to have a profit of $741 (Canadian dollars) ha^{-1}. In contrast, the infected unamended control plots were estimated to have a loss of $615 in 1996 and of $26 in 1997. In the year of application (1996), treatment with meat and bone meal resulted in a loss of $15,561 ha^{-1}. The high cost of meat and bone meal ($360 t^{-1}) accounted for the dramatic increase in production costs. Further, growth and yield of potato were reduced because of residual phytotoxicity in soil due to the amendments. In 1997, the year following treatment, an average profit gain of $462 ha^{-1}

was estimated. In 1996, poultry manure resulted in an average profit of \$926 ha^{-1} and in 1997, of \$731 ha^{-1} (estimated). An increase in income and reduced production costs accounted for the large profit estimated with poultry manure amendment. Both total and percent marketable yield increased due to reduced disease levels. Furthermore, poultry manure lowered production costs since it was a substitute for fertilizer and preplant-incorporated herbicide. Organic amendments should be considered to have many potential benefits for crop yield and quality. The use of poultry manure seems to be a practical option to control soilborne diseases in Ontario potato fields. However, a rise in the cost of poultry manure or a shortage in supply might prevent adoption of poultry manure amendment as a control option.

Use of Soil Solarization and Biofumigation for the Control of *Sclerotium cepivorum* in Southern Italy

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Sclerotium cepivorum, the causal agent of white bulb rot of *Allium* spp., represents a major limiting factor to onion crops in Calabria. In a previous experiment, soil solarization (SoSo) treatment carried out for 45 days, from July to August, reduced significantly the disease incidence and increased the total marketable yield of onion cropped in naturally infested soils, when compared with the untreated control. During summer 1997, the effect of SoSo alone or combined with a soil amendment consisting of chicken manure at 1 kg/m^2 (biofumigation), upon the mortality of sclerotia of *S. cepivorum*, was examined in field plot experiments. The treatments were carried out for 12, 27 and 47 days by covering the soil with three different plastic mulches: low density polyethylene sheet (LDPE), black gas virtually impermeable film (VIF), or black coextruded ethylenevinyl acetate-black PE film (COE). Biofumigation carried out by mulching soil with PE film achieved complete kill of the sclerotia of *S. cepivorum*, buried at the two tested depths, after both 12 and 27 days. The addition of chicken manure to the soil enhanced significantly, as soon as 12 days after treatment, the sclerotia mortality at the two tested depths. Both black plastic mulches (COE and VIF) had a lesser effect on sclerotia mortality, when compared with the transparent mulch. The increase in sclerotia mortality at the 15 and 30 cm depths, induced by biofumigation by means of COE film when carried out for 47 days, has potential for improved disease control.

In conclusion, field solarization of organic-amended soil resulted in better control of sclerotia of *S. cepivorum* and shortened the application time from that of either treatment alone.

Soil Solarization Using Sprayable Plastic Polymers as Plastic Mulch

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Soil solarization (SoSo) is a modern approach to soil disinfestation. It consists of solar heating tarped, moist soil during the hot summer months. Solarization is of special importance as a soil disinfectant in todays intensive agriculture, since the system is simple to apply and does not necessarily involve hazardous chemicals or complicated machinery. Nevertheless, applying plastic films for soil mulch requires special mulching equipment and involves special procedures. The edges should be buried to hold the film in place, and in continuous mulch treatment the sheets are glued together. It is not possible to change the mulching pattern during work, and capacity of area coverage is limited. Plastic mulches must be removed from the fields before crop growth or after harvest; removal is expensive and labor-intensive. Plastic residues are often left in the field and can cause problems to agricultural practices and machinery of future crops.

Soil mulch for SoSo using spray application of degradable polymers offers a feasible and cost-effective alternative to plastic tarps. The polymer is applied by spraying the desired quantity. The polymer composition forms a membrane film which can maintain its integrity in soil and elevate soil temperature. Nevertheless, the membrane is porous and allows overhead irrigation. In experiments it was found that certain spray mulches were able to raise soil temperature and retain soil moisture under summer conditions. Soilborne populations of phytopathogenic fungi were significantly reduced by the process of solarization. SoSo using this procedure was as effective as solarization using plastic film, in controlling Verticillium wilt and common scab in potato, and peanut pod wart in peanut, during two consecutive crops. Sprayable polymers for SoSo enable combining solarization with fumigants at reduced dosage, or organic amendments (bio-fumigation), for improved pest control.

A Case Study: Nonchemical Disinfection of Peanut Seeds from Soilborne Pathogens

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Contaminated seed stock is an important source of disease dissemination to new areas. Production of healthy, pathogen-free seeds is, therefore, a continuous task. Peanut is a cash crop, grown frequently in certain areas of Israel. Rapid build-up of seed- and soilborne diseases, such as those caused by *Aspergillus niger, Rhizopus stolonifer, Pythium* spp. and *Verticillium dahliae* in peanut fields is, therefore, a major problem. Heavy metham-sodium treatments are used regularly against these diseases. A relatively new peanut disease, peanut pod wart, caused by *Streptomyces* sp., was recorded a few years ago in peanut fields in the main growing area of this crop, and has since spread rapidly. It was proposed that continuous application of metham-sodium enhanced the rapid spread. Control of this disease, as of most soilborne diseases, is complicated and expensive. The demand for a technology to prevent introduction of seedborne inoculum was therefore evident.

Preliminary studies have shown that peanut seeds were not contaminated when collected aseptically from diseased pods. After mechanical cracking, however, pronounced contamination of the seed was recorded. Low-volume spray treatment of the pods with 3% iodine dissolved in glacial acetic acid (to receive a cover density equal to or higher than 500 droplets cm^{-1}) almost eliminated the *Streptomyces* population in wart tissue. Despite being effective and relatively non-toxic, this treatment caused operational problems due to its unpleasant smell, which necessitated the use of gas masks during application. This, and the public interest in non-chemical control methods, led to an attempt to develop a non-chemical disinfection method. As the pod has a thick shell-wall, and an air gap between the inner side of the shell and the seeds, it was assumed that a brief exposure of the shell to open flame might control the *Streptomyces* without damaging the seeds. It was found that a 4-sec exposure of diseased pods to butane-air flame (at 740°C) reduced the *Streptomyces* population in the wart tissue by 10^4. A 6-sec exposure did not affect germination. Contamination of the seeds by molds (*A. niger* and *R. stolonifer*) was also reduced. Populations of possible antagonists to soilborne fungi such as *Trichoderma* sp. were also reduced, but to a lesser extent. When the flaming system was installed right before the cracking machine, an increase of 25-35% in seed production was recorded. Peanut pod flaming was introduced very rapidly in Israel. Only 6 years after the first semi-commercial plot was established, *ca* 60% of all the peanut seeds produced in Israel are flamed during production. This has led to both improved quality and increased seed quantity produced from identical quantities of pods.

Control of Fusarium Wilt by Methyl Bromide in Greenhouse-Grown Carnations in Monoculture

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The effectiveness of methyl bromide (MB, 80 g m^{-2}) in controlling *Fusarium oxysporum* f.sp. *dianthi* (*F.o. dianthi*) was studied at soil depths of 0–60 cm, subdivided into three layers, *viz.*, 0–20, 20–40 and 40–60 cm, in four carnation greenhouses during four successive growth seasons. Each growth season, the number of propagules of *F.o. dianthi* in the soil was counted before and after fumigation. The efficiency of MB control of the pathogen was also measured in terms of the viability of *F.o. dianthi* propagules placed in the soil at depths of 0, 10, 20, 30, 40, 50 and 60 cm. Carnation cultivars (*Dianthus caryophyllus*) highly susceptible to Fusarium wilt were planted in each of the four greenhouses. At the end of each growth season, the largest number of propagules (57–100% of the total count) was found in the top soil layer, 0–20 cm; less inoculum was found in the 40–60-cm layer and least in the 20–40 cm. MB reduced the *F.o. dianthi* propagule count at all soil depths between 0 and 60 cm, but was most effective in the upper 30 cm. A count of *F.o. dianthi* propagules in the fumigated greenhouses on the ten occasions when their level at the end of the previous growth season was high, revealed propagule survival rates of 0% (below detection level) five times, 0.1% twice, 0.6% once, 1.6% once and 4.6% once. Nevertheless, at the end of each growth season, both inoculum level in the soil and Fusarium wilt incidence were high even when propagule counts in the 0–60 cm soil layer were below the level of detection. Vertical distribution of propagules in the 120-cm soil profile in four commercial greenhouses revealed that the largest count of propagules was in the 0–20-cm layer but a few propagules were found to a depth of 120 cm. Incidence of wilt when inoculum was placed 60–75 cm deep was 22%, as compared with 54% when inoculum was placed in the top 0–30 cm. These findings could indicate that failure to reduce Fusarium wilt in carnations by MB due not only to the low number of propagules which survived fumigation, but also to an accumulation of inoculum, *via* infected roots, at soil depths where fumigation is ineffective.

Detection of *Pythium* by Serological and Molecular Methods

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During the last decade much progress has been made in the detection of *Pythium* to predict and quantify disease. A method based on a polyclonal antibody (PAb) which enables detection of the carrot cavity spot pathogen (*Pythium violae*) before seed is sown, has been fully commercialized. The test allows growers to make a rational choice of fields in which to grow carrot, and also whether or not to use fungicide(s) for control of the disease. Many fields are not at significant risk of cavity spot and the cost of fungicides may be saved. Some fields have sufficient evidence of *P. violae* to make it imperative to apply fungicides, and a small number have such high disease levels that carrot should not be grown.

Despite the commercial success of this cavity spot test based on a PAb, there is a risk of false positive results by showing the presence of non-cavity spot pathogens. In the UK this has not been a significant problem because of the habit of *P. violae*, a fungus with a temperature optimum below 20°C, for making explosive growth in the winter, when other *Pythium* species are dormant. However, in other countries, the cross-reactivity of the PAb with other species of *Pythium* could be a real problem. Neither PAbs nor monoclonal antibodies (MAbs) to other species of *Pythium* have shown sufficient specificity to be used in practical detection tests.

The advent of molecular biological methods has given us completely new opportunities both to identify and to detect members of this difficult genus, that detection being species-specific. For identification, the use of PCR/RFLP of the internal transcribed spacer region (ITS) of rDNA with just four endonucleases (*Cfo*I, *Hinf*I, *Mbo*I and *Taq*I) gave restriction fragment patterns characteristic to species. There was little intra-specific variation. Sequencing of the ITS of a large number of species also made possible specific detection, because primers unique to single species could be identified. Using variations on PCR it has been possible to demonstrate the extremely low levels of detection possible (three orders of magnitude better than the competition, ELISA) in laboratory studies. The system has made possible specific detection of *P. aphanidermatum* in nutrient solution growing systems, and has some possibility of quantifying inoculum. For *Pythium* in soil there are likely to be more technical difficulties in getting a preparation method which works and is not too complicated or expensive to use.

Virulence and VCG Diversity in *Verticillium* spp. from Plant Tissue and Soil

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Vegetative compatibility was studied in three soilborne species of *Verticillium*: *V. dahliae*, *V. nigrescens* and *V. tricorpus*, using nitrate-nonutilizing (*nit*) mutants. *V. dahliae* is a strong wilt-inducing pathogen, whereas the two other species are considered weak pathogens or saprophytes. Three vegetative-compatibility groups (VCGs) were found among several hundred isolates of *V. dahliae* from 13 host-plant species and soil, at 45 sites in Israel, and identified as VCG2A, VCG2B, and VCG4B by using reference strains of the OARDC international system: 26 isolates were assigned to VCG2A, 128 isolates to VCG2B, and 375 to VCG4B. Each VCG has its specific geographical distribution: all VCG2B isolates were recovered from the northern part of Israel and all VCG4B isolates - from the south. Some overlap was observed in the central region. Isolates of the minor VCG2A were scattered among the two major VCGs, ranging from 8% (north) to 3% (south) of the isolates tested. Isolates of the same VCG are more similar to one another, in some properties including pathogenicity, than isolates from different VCGs. Different pathotypes were found among 65 isolates tested, using cotton ('Acala SJ-2') and eggplant ('Black Beauty') as differentials. VCG2A and most of the VCG4B isolates, irrespective of their origin, induced weak-to-moderate symptoms on cotton and moderate-to-heavy symptoms on eggplant. Two cotton isolates of VCG4B induced heavy disease on both differentials. On the other hand, VCG2B is more heterogeneous and several pathogenicity patterns were revealed among its isolates. All cotton isolates of VCG2B caused severe foliar symptoms, strong stunting and often death of inoculated cotton plants, but induced only weak-to-moderate symptoms on eggplant. VCG2B isolates from other hosts induced more severe disease on eggplant than on cotton, manifesting the presence of subgroups which vary in their aggressiveness to these differentials.

Isolates of *V. tricorpus* were recovered from potato, weeds and soil from seven locations, and isolates of *V. nigrescens* were isolated from cotton, eggplant, potato, weeds and soil from nine locations throughout Israel. Nine VCGs were established among 26 isolates of *V. tricorpus* and 11 VCGs among 19 isolates of *V. nigrescens*. Local populations of *V. nigrescens* and *V. tricorpus* were more heterogeneous than those of *V. dahliae*: when two or more isolates were recovered from a given site (whether from plants or soil), they often belonged to more than one VCG. In contrast, more than 90% of the *V. dahliae* population in the northern region of Israel belongs to VCG2B, whereas over 90% of the population in the southern region belongs to VCG4B, manifesting a high level of uniformity in each region. These results manifest apparently greater diversity in the weakly pathogenic and soil saprophytic *Verticillium* species as compared with that observed in the strongly pathogenic *V. dahliae*. Isolates in the same species differed from one another in radial growth rate, pigmentation, and temperature responses. Members of the same VCG were more uniform, possibly indicating clonal origin. There was some association of VCGs with their source of origin: five VCGs of *V. nigrescens* were recovered from plants only, whereas six were recovered from soil only. In *V. tricorpus*, two VCGs were recovered from both plants and soil, whereas two others were isolated from soil only, and five from plants only. All tested strains of *V. tricorpus* and *V. nigrescens* were nonpathogenic to cotton and eggplant in greenhouse experiments, causing neither leaf damage nor marked stunting of inoculated plants, although they were able to colonize roots and the hypocotyl of such plants.