

**BIOLOGICAL CONTROL OF BACTERIAL WILT IN
TOBACCO CAUSED BY
*Ralstonia solanacearum***

by

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I the undersigned hereby declare that the work contained in this thesis for the degree of Master of Science in Agriculture is my own original work and has not previously in its entirety, or in part, been submitted at any university for a degree. I assign all copy rights to this thesis to the University of the Free State.

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GENERAL INTRODUCTION

This thesis consists of three separate research studies compiled in a supportive sequence. The literature study, Chapter 1, reviews published information on the importance of the ecology of soilborne pathogens within the rhizosphere. With the emphasis on bio-fumigation, all major biological control strategies are compared and contrasted. To put the potential of biological control strategies of soilborne pathogens in perspective, all host-, environment- and pathogen related factors influencing the success thereof, are also discussed.

In Chapter 2, series of summer- and winter crops were evaluated for rhizosphere suppression of *Ralstonia solanacearum* (Race 1, Biovar III). All trials were executed in a greenhouse. The test crops were cultivated in soil with a predetermined, high population of the pathogen. After two to four months of growth, the pathogen populations present in the rhizospheres of each crop were quantified, and statistically compared with each other, as well as with the initial pathogen population of the soil. The results obtained served as a screening test for possible crops to be used in crop rotation systems for the biological control of bacterial wilt of tobacco caused by *R. solanacearum*.

To test the crops with rhizosphere suppression abilities of *R. solanacearum* in practice, crop rotation trials were designed and executed over a period of four years. Chapter 3 reported on the greenhouse and field trials, which proved that *Tagetes spp.* (marigolds) in combination with non-host winter crops not only resulted in lower disease incidence in the follow-up susceptible tobacco, but also resulted in a 60% higher yield when followed with a resistant tobacco cultivar.

**LITERATURE REVIEW OF
BIOLOGICAL AND CULTURAL STRATEGIES FOR THE CONTROL OF
SOILBORNE PLANT PATHOGENS WITH SPECIFIC REFERENCE TO BIO-
FUMIGATION AND SOIL AMENDMENTS**

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**BIOLOGICAL AND CULTURAL STRATEGIES FOR THE CONTROL OF
SOILBORNE PLANT PATHOGENS WITH SPECIFIC REFERENCE TO BIO-
FUMIGATION AND SOIL AMENDMENTS**

1.0 INTRODUCTION

1.1 Ecology of soilborne pathogens

Baker and Cook (1982), described the biological world as a vast interacting network of living populations in a state of dynamic equilibrium, reflecting changes in their physical environment and their relationship to each other. Soilborne pathogens form part of this world. During its saprophytic phase, the soilborne root pathogen shares the same terrestrial environment as other soil microorganisms (Griffin, 1985). Although man has spent more time and research on the interactions of soilborne pathogens and their hosts than on the ecology of these microorganisms the following points have been established (Baker & Cook, 1982):

- Soilborne pathogens producing metabolic by-products (antibiotics) that inhibit competitors for a given ecological niche have an advantage.
- Some soilborne pathogens occupy a site during warm or moist periods and become dormant during the cool or dry part of the year.
- A pathogen will increase until the limitations imposed by the biotic and abiotic environment counterbalance the rate of increase.
- Slight changes in one or more factors may exert a profound effect on soil microorganisms.

- Parasites are at a greater competitive disadvantage than saprophytes when they are outside their host.
- Ability to invade and colonize organic debris may improve opportunities for infection of a host by providing a necessary food base.

1.2 The Rhizosphere Interactions

rhīz ' o - comes from Greek - *rhiza* = a root.

sphēre = field of action, influence or existence; natural surroundings.

The term *rhizosphere* was used for the first time in 1904 by Hiltner to describe the interaction between bacteria and legume roots (Lynch, 1990). Today we recognize the importance of this milieu, and have redefined the definition thereof. In broad, it refers to the volume of soil influenced by the roots (Campbell & Greaves, 1990). The different components of the rhizosphere are also recognized as the *endorrhizosphere* – the cell layers of the root itself where microorganisms can colonize; the *ectorrhizosphere* – the area surrounding the root; and the *rhizoplane* – the root surface (Campbell & Greaves, 1990; Lynch, 1990).

The quantities and types of substrates in the rhizosphere are different than that in the bulk soil. This leads to colonization by bacteria, fungi, protozoa and nematodes. An interacting trinity of the soil, the plant and the organisms associated with the roots, determines the rhizosphere environment. The interaction between the roots and the organisms can be beneficial, neutral or harmful. The rhizosphere can be manipulated to increase the balance of beneficial over harmful effect (Lynch, 1990).

It was only in the 1930s that researchers started to recognize the significance of the rhizosphere in soilborne diseases when the activities of the microflora in the rhizosphere of wheat affected by the take-all fungus were studied. Scientists started to understand the finely balanced equilibrium of harmless, beneficial and pathogenic microorganisms in the rhizosphere, susceptible to a whole range of plant-mediated influences (Hornby, 1990).

1.3 Control of soilborne pathogens

Since the beginning of agriculture, generations of farmers have tried to develop practices for combating plagues suffered by their crops. A growing understanding of the interaction of pathogen and host has enabled the development of various methods for the control of specific plant diseases. Like all other diseases, the control of soilborne pathogens is based on the traditional principles of plant disease control strategies formulated as early as 1929 (Plant Disease Management Strategies – *website*).

- **Avoidance** - select time of year, or site where there is no inoculum or where environment is unfavorable for infection.
- **Exclusion** - prevent introduction of inoculum
- **Eradication** - eliminate, destroy or inactivate inoculum
- **Protection** - prevent infection by means of a barrier (prophylactic, toxic chemical)
- **Resistance** - plant resistant or tolerant cultivars
- **Therapy** - Cure plants that are already infected

These strategies can be employed in control methods that can be classified as regulatory-, cultural-, biological-, physical- and chemical control. (Agrios, 1997c)

Cultural control methods strive to create conditions that are unfavorable for the pathogen to multiply, thus reducing the population, and thus avoiding contact with the plant (Agrios, 1997c). The major constraint on the multiplication of soilborne pathogens in soil is the limited supply of utilizable energy sources (Lockwood, 1990).

Biological control rarely eliminates a pathogen from the soil, usually it reduces its numbers or ability to induce disease (Baker & Cook, 1982). Cook & Baker (1989b) defined biological control of plant pathogens as: *The reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man.* If biological control has not been operating in nature, none of the wild plants susceptible to various pathogens would have survived (Baker & Cook, 1982). In nature the balance is maintained through a network of intricately interacting organisms and the abiotic environment. The principles of biological control prevail in nature, therefore pathogens are not eliminated, and only their activities are curbed (Baker & Cook, 1982).

2.0 BIOLOGICAL CONTROL STRATEGIES

2.1 Bio-fumigation and soil amendments

Many factors are involved during the decomposition of amendments and plant residues in the soil. There is a fast growing interest in disease suppression due to the influence of volatile material that evolves during decomposition of these amendments on the pathogen (Lewis & Papavizas, 1970). Biofumigation refers to the suppression of soilborne pests and pathogens by biocidal compounds, principally isothiocyanates (ITC's), released from Brassicaceous rotation and green manure crops when glucosinolates (GSL's) in their tissues are hydrolyzed (Kirkegaard & Matthiessen, 1999; Matthiessen & Kirkegaard, 2006). The bioactivity of various ITC's released by *Brassica* tissues is well known and published. Because many ITC's are volatile, the term *biofumigation*, was used for the first time in 1993 to describe the suppression of soilborne pests and pathogens by *Brassica* crops (Matthiessen & Kirkegaard, 1998; Matthiessen & Kirkegaard, 2006). GSL's are relatively inactive against microorganisms, but when the tissue of these Brassicaceae are disrupted, GSL's are hydrolyzed by endogenous myrosinase (thioglucoside glucohydrolase EC3.2.3.1) to release ITC's, thiocyanates, nitriles or oxazolidinethiones (Sarwar, & Kirkegaard, 1998; Sarwar, Kirkegaard & Wong, 1998; Kirkegaard & Sarwar, 1999). According to these authors, environmental conditions and the type of side chain (which can be aliphatic, aromatic or indolyl) on the parent molecule will determine the nature of the hydrolysis product.

In vitro studies: *In vitro* studies by Sarwar *et al.*, (1998) compared the relative toxicity of ITC's to five different soilborne fungal pathogens when exposed to ITC's dissolved in agar. Although all ITC's (aromatic and aliphatic) suppressed all the fungi, there was a definite difference in the sensitivity of the pathogens towards the ITC's. *Gaeumannomyces graminis var. tritici*, was the most sensitive to the ITC's, *Fusarium graminearum* and *Rhizoctonia solani* were intermediate, *Bipolaris sorokiniana* was less sensitive and *Pythium irregulare* showed a high degree of resistance to the ITC's.

Kirkegaard, Wong & Desmarchelier (1996) reported on the effect of volatile compounds released from different tissue types and ages of different *Brassica* species. Roots and shoots of field-grown canola (*Brassica napus* cv. Oscar) and Indian mustard (*Brassica juncea*, 99Y-1-1) were sampled at flowering and harvest maturity. Seed meal of the same varieties was also obtained. These plant tissues were hydrolyzed and tested *in vitro* for the suppression of five soilborne pathogenic fungi that affect cereal yields - *Gaeumannomyces graminis var. tritici*, *Fusarium graminearum*, *Rhizoctonia solani*, *Bipolaris sorokiniana* and *Pythium irregulare*. Concentrations of the volatile compounds released from each of these tissues were analyzed using headspace chromatographic analysis. The results from this study confirmed that: (i) all isolates were suppressed by all tissue types, however *Gaeumannomyces* was once again the most sensitive and *Pythium* the most resistant; (ii) all tissue types at flowering were more suppressive than at maturity; (iii) there were significant differences in suppression between tissue types - with mustard shoots and –seed meal the highest and canola shoots the least suppressive. Mustard roots and canola roots were intermediate. The ITC

concentrations measured in the headspace were closely related to the degree of fungal suppression by different tissue types. HPLC chromatograms and gas chromatography mass spectrometry performed, concur that glucosinolate profiles from several cruciferous plants indicated major differences between the seed, leaf and root profiles (Angus *et al.*, 1994; Sang *et al.*, 1984).

Greenhouse & field studies: Brassicas are recognized as “break crops” due to the fact that they are used to break the life cycle of serious soilborne pathogens such as *Gaeumannomyces graminis* var. *tritici* which causes take-all of wheat. (Angus, *et al.*, 1994). Brassica plants were screened in Queensland (Australia) for the first time for the control of the soilborne, plant pathogenic bacterium, *Ralstonia solanacearum* (Akiew, Trevorrow & Kirkegaard, 1996). In a greenhouse trial, the soil population of this pathogen declined when decaying residues of mustard (*Brassica juncea*) and canola (*Brassica napus*) were added to the soil. Concurring with the *in vitro* testing, the mustard residues reduced the pathogen population from 10^7 colony-forming units (CFU) to an undetectable level, whereas canola only suppressed the pathogen population to 40% of the untreated control. Tomato plants, a host to *R. solanacearum*, had a 100% survival when planted in pots with *R. solanacearum* infested soil amended with mustard residues. Canola amendments only resulted in a 53% survival of the susceptible tomato plants. This trial was repeated in the field under normal conditions, where mustard and canola reduced disease incidence by 59% and 28% respectively. According to Arthy, *et al.* (2002), the same tendency of protection was bestowed by Brassica amendments on susceptible tobacco plants in *R. solanacearum* infested soil.

Alternative for chemical fumigation: Soil sterilization for the control of nematodes and other soilborne pathogens by means of chemical fumigation is still to this day the most effective control method (Agrios, 1997c). The most frequently used, and thus fumigants with the best ability to clean up soil, are methyl bromide, dichloropropene, chloropicrin, dazomet, metam sodium and ethylene dibromide (EDB) (Agrios, 1997c; Eddy, 1999). Methyl bromide, developed and produced since 1932, undoubtedly the best of all, had to be phased out by 2005 in all developed countries due to its negative effect on the environment and the ozone layer (Eddy, 1999; Matthiessen & Kirkegaard, 2006). Although these chemicals provide great advantages to agriculture, most of them have proven side effects on nature and will eventually be phased out (Gamliel & Stapleton, 1995). Due to these facts, there is considerable interest in biofumigation as an alternative to synthetic soil fumigants in horticulture and broad acre agriculture (Matthiessen & Kirkegaard, 1998; Sarwar *et al.*, 1998; Matthiessen & Kirkegaard, 2006)

Other biofumigant agents: Although the term biofumigation was first used, and always referred to the biocidal effects of isothiocyanates (ITC's) released from Brassicaceous plants (Matthiessen & Kirkegaard, 1998) it can, and should be extended to other volatile bio-substances with the same qualities exuded from other plant species. Thiophene, a heterocyclic, sulphurous compound with strong biocidal activity, can be extracted from the undisturbed rhizosphere of *Tagetes patula* and *Tagetes erecta* (Croes *et al.*, 1989; Jacobs *et al.*, 1994; Tang, Wat & Towers, 1987; Terblanche & de Villiers, 1997). This natural volatile, biocide, with a higher concentration in the roots than in the rest

of the plant (Jacobs *et al.* 1994), can thus also be used for biofumigation. Suppression of nematodes by *Tagetes spp.* is widely known and published (Schepman & Jansen, 1994; Ploeg, 2002). According to Caswell *et al.*, (1991) root secretions from the undisturbed rhizosphere of *T. patula* are toxic to the reniform nematode. Alexander & Waldenmaier (2002) significantly reduced the population of *Pratylenchus penetrans* in the soil and the roots of susceptible tomato and potato plants when they double cropped with *Tagetes erecta* L. In Canada the population density of *P. penetrans* was also reduced to levels below the economic threshold for tobacco production when *T. erecta* and *T. patula* were cultivated as an alternative to chemical fumigation for nematodes (Reynolds, Potter & Ball-Coelho, 2000).

Improving efficacy of biofumigation: According to Gamliel & Stapleton (1995) the pathogen sensitivity to volatile compounds was increased as soil temperature increased. Pathogen control in the solarized-amended soil attributed to a combination of thermal killing and generation of biotoxic volatile compounds.

*(For reported soilborne pathogens controlled by biofumigation and biofumigation in combination with solarization, see **Table 1..**)*

2.2 Other Bio- and Cultural Control Strategies

2.2.1 Beneficial Microbial Interaction

The roots of most plants have a symbiotic relationship with certain beneficial microbes that exist as mycorrhizae. These mycorrhizae colonize

roots intercellularly or intracellularly and obtain organic nutrients from the plant. This interaction is beneficial to the plant due to the fact that the mycorrhizae enhance nutrient uptake and water transport by the plant, thus increasing growth and yield. These mycorrhizae can also protect the host plant against several soilborne pathogens (Agrios, 1997c). Beneficial bacteria with rhizosphere competence (e.g. *Pseudomonas*, *Enterobacter* and *Azospirillum*) have been known since 1978. However beneficial fungi like *Trichoderma*, with high rhizosphere competence, is a more recent discovery (Harman & Lumsden, 1990).

The interaction between the mycorrhizal organisms and other possible deleterious organisms is complex, and the ways soilborne pathogens can be influenced can include (Reid, 1990):

- competition for nutrient uptake or actual infection sites
- alteration of the physiology of the host plant
- formation of physical barriers to infection by sheathing mycorrhizas
- production of toxic or inhibitory compounds
- enhancement of nutrient uptake by the plant to compensate for damage to roots by disease

2.2.2 Antagonist and Avirulent Mutant Competition

Antagonists are rhizosphere organisms capable of affecting the plant or other microorganisms operating in a dynamic environment of enhanced microbial activity. These antagonists may exhibit all principal forms of antagonism like antibiosis, competition, parasitism and predation (Hornby, 1990). Several non-plant pathogenic fungi have the ability to invade structures

of phytopathogenic soil fungi such as *Pythium*, *Phytophthora*, *Rhizoctonia*, *Sclerotinia* and *Sclerotium*. These pathogens are then parasitized (**mycoparasitism**) or lysed (**mycolysis**) by these mycoparasitic fungi. These mycoparasitic fungi, as well as pseudomonad and actinomycetous bacteria, can also infect the resting spores of soilborne pathogenic fungi (Agrios, 1997c).

Very few attempts to utilize antibiotics and competition for the control of soilborne pathogens in the field have been successful (Hornby, 1990). The best results in biological control of soilborne pathogens with these antagonistic microorganisms are usually obtained when the antagonist is introduced in treated and nearly sterile soil in a glasshouse. Applications of biological control with microorganisms under field conditions are usually through management of resident antagonists in the untreated field soil (Cook & Baker, 1989a).

The most common mycoparasitic fungi are *Trichoderma* spp., which have shown to parasitize mycelium of *Rhizoctonia* and *Sclerotium*. *Trichoderma* spp. have by inhibition of *Pythium*, *Phytophthora*, and *Fusarium* reduced the diseases caused by these pathogens (Agrios, 1997c). *Trichoderma* spp. and *Didymella exitialis* decreased the disease severity caused by the take-all fungus by 50% under sterile conditions in the glasshouse (Hornby, 1990).

Biological control can also be obtained when an avirulent strain or mutant of the pathogen can become attached at receptor sites on the host cell, in which case the sites are no longer available to the pathogen (Cook & Baker, 1989b). This is an effect of protection, interference or physical blockage. This biological control is an example of *cross protection*, achieved with an avirulent strain acting as a competitor of the pathogen resides inside tissues of the host plant (Cook & Baker, 1989b).

2.2.3 Crop Rotation

Crop rotation is the oldest and best-known example of biological control, because it generally lowers the inoculum density of the pathogen (Baker & Cook, 1982). Many diseases build up in the soil when the same crop, or closely related crops, are grown in the same field year after year. Regular rotation of crop species can break this cycle. The population of a soilborne pathogen can be reduced by not planting crops belonging to species or families that are attacked by this particular pathogen. If the pathogen is a *soil invader*, surviving only on living plants or as long as host residues are present in the soil, satisfactory control can be achieved with a 3 or 4 years crop rotation period. However, in the case of *soil inhabitants*, which produce long-lived spores, or can survive as saprophytes for 5 to 6 years, crop rotation is less effective. The efficacy of disease control by crop rotation thus depends mainly on the life cycle and behavior of the pathogen (Agrios, 1997c; Lucas, 1975).

The crop succession or crop combinations must be carefully chosen according to the causal soilborne pathogen's host status. The occurrence of two or more species of *Meloidogyne* in the same field complicates the selection of rotation crops, because the reproduction of different *Meloidogyne* species varies not only with crops, but also with cultivars. The selection of crops must thus be based on host resistance to the more aggressive species and their ability to shift populations from more aggressive to less aggressive species (Fortnum & Currin, 1993; Fortnum, Lewis, & Johnson, 2001). The incidence of take-all on wheat, caused by *Gaeumannomyces graminis* var. *tritici*, was significantly lower and the grain yield higher after rotation with non-host, non-

cereal crops whereas the disease was more severe after rotation with non-host, cereal crops (Kollmorgen, Griffith & Walsgott 1983)

Various soilborne diseases are managed and controlled by the traditional and most widely used method – crop rotation. Well planned crop rotation can successfully control nematode populations in tobacco fields, resulting in an increased tobacco yield (Fortnum, Lewis, & Johnson, 2001) *Phytophthora nicotianae* var. *nicotianae*, which causes black shank of tobacco, can survive in the soil for as long as 8 years (Lucas, 1975), and is therefore difficult to control. However, de Villiers reported in 1987 that plots planted to blue buffalo grass (*Cenchrus ciliaris*) for three years, showed a significant lower incidence of black shank when rotated with tobacco. Rotation plays a major part in the incidence of take-all in wheat. The wheat take-all fungus, which is probably the most serious fungal disease of wheat, can be successfully controlled when the wheat is rotated with medic, peas and other non-cereal crops, (Kollmorgen, Griffith, & Walsgott, 1983; Rovira & Venn, 1985). Although to a lesser extent than take-all, root diseases on wheat caused by *Rhizoctonia solani*, are also less after rotation with medic and peas (Rovira & Venn, 1985).

The efficacy of crop rotation can be enhanced by combining it with other disease control methods as part of an integrated disease management system. When the correct crop sequence, soil fumigation, resistant cultivars, standard cultivation methods and proper sanitation are intelligently combined, crop losses due to diseases will be minimized (Lucas, 1975). The application of the nematicide 1,3-dichloropene in a series of rotation systems, increased tobacco yields and reduced root galling across all the rotation crops (Fortnum, Lewis & Johnson, 2001). Rovira and Venn (1985) also proved that successful control of

take-all on wheat with crop rotation can be enhanced when standard cultivation practices are used. However, conservation tillage (reduced tillage or direct drilling) in a rotation with non-cereal crops, will not have this beneficial effect on the control of take-all on the follow-up wheat planting.

2.2.4 Soil Solarization

Soil solarization, also referred to as *solar heating*, *soil pasteurization* or *soil tarping*, is a non-chemical, soil disinfestation method that utilizes solar energy for heating the soil. This is achieved by covering (mulching / tarping) the soil with transparent polyethylene sheets during the hot season. The polyethylene sheets should not be thicker than 25-30 μ m. The polyethylene sheets laid on the soil, serve as traps for capturing solar energy, increasing the soil temperature to levels detrimental to plant pathogens (Chase, Sinclair & Locascio, 1999; Chellemi *et al.*, 1997; Katan, 1985). Soil solarization involves hydro-chemical processes that lead to physical, chemical and biological changes in the soil. These changes take place during and even after termination of solarization (Hardy & Sivasithamparam, 1985). Different to most soil sterilization techniques, soil solarization targets only meso-phyllic organisms, which include most plant pathogens and pests, without destroying the beneficial mycorrhizal fungi, *Trichoderma spp.*, growth-promoting *Bacillus spp.* and fluorescent *Pseudomonas* (Pinkerton *et al.*, 2000; Stapleton & De Vay, 1983 & 1984). Soil solarization is non-chemical, safe, and has the advantage to be included in an integrated pest management system in order to enhance biological control approaches (Pinkerton *et al.*, 2000). It is also an economical alternative to chemical soil fumigation (Chellemi *et al.*, 1997)

Solar heating is carried out at relative low temperatures compared to steam sterilization and is, therefore, less drastic on living and nonliving soil components. Most saprophytes can survive temperatures reached during soil solarization, and are thus successful in occupying the available niches created by soil solarization (Greenberger, Yogev & Katan, 1987). Results published by Bendavid-Val *et al.* in 1997 suggested that soil solarization only affects the vigour of the arbuscular mycorrhizal propagules and does not eradicate the fungal population thereof. Hence the return of the arbuscular mycorrhizal vigour after 5-6 weeks of host plant growth. Rapid soil reinfestation by the pathogen does not occur because a biological vacuum is not created like with steam sterilization or chemical fumigation (Hardy & Sivasithamparam, 1985; Katan, Fisher, & Grinstein, 1983). Long-term disease control was confirmed in Israel where one soil solarization treatment, depressed *Fusarium* wilt in a cotton field (Katan, 1985) and white root rot caused by *Dematophora necatrix* in an avocado orchard (Lopez-Herrera *et al.*, 1998.) for three years.

Increased growth response of the crop following soil solarization is often reported. Under optimal conditions soil solarization decreases or even controls a variety of soilborne fungi (Stapleton & De Vay, 1984), bacteria (Antoniou, Tjamos & Panagopoulos, 1995) and nematodes (Pinkerton *et al.*, 2000) – see **Table.1**. The control of these plant pathogens and consequently disease reduction, frequently results in an increased plant growth response and therefore a yield increase (Bendavid-Val *et al.*, 1997; Stapleton & De Vay, 1982 & 1984). Cotton yields increased for three consecutive years after *Fusarium* wilt was depressed by soil solarization (Katan, Fisher & Grinstein, 1983). Seed

cotton yields increased up to 130.9% after the population of *Verticillium dahliae* had been reduced by soil solarization (Melero-Vara *et.al.*, 1995)

Soil solarization can only be effective in areas with prevailing high temperatures and intense solar radiation (Ahmad, Hameed & Aslam, 1996). The soil should be kept moist to improve heat conduction. The mulching period should be four weeks or more to achieve results in the deeper soil layers. Thermal inactivation of the pathogen depends on the thermal dose - a product of both the temperature and exposure time which are inversely related (Pinkerton *et al.*, 2000; Katan, 1985). Because the efficacy of soil solarization is totally dependent on solar energy, factors like cloud cover, day length and rain which interfere with the maximum day temperature and the duration thereof, will result in ineffective control of soilborne plant pathogens (Chellemi & Olson, 1994; Coelho, Chellemi & Mitchell, 1999). In North Florida soil solarization was not as effective as methyl bromide to reduce *Phytophthora* sp. populations in the deeper soil layers. It was speculated that where the rainy season coincided with the high summer temperatures, soil solarization would not be successful (Coelho, Chellemi & Mitchell, 1999)

In solarized plots, where effective disease control is obtained after six to eight weeks, the maximal soil temperatures achieved, range from 45-53°C at a depth of 5-10cm and 38-45°C at 20-25 cm. (Chellemi *et al.*, 1997; Coelho, Chellemi & Mitchell, 1999; Katan, 1985; Katan, Fisher & Grinstein, 1983; Lopez-Escudero & Blanco-Lopez, 2001; Porter & Merriman, 1985). In India the temperature of plastic mulched, irrigated soil went as high as 57°C at depths of 0-15 cm and 50°C at depths of 16-30 cm. This was achieved in a hot arid region with intense solar radiation (Lodha, Sharma & Aggarwal, 1997)

3.0 FACTORS INFLUENCING BIOLOGICAL CONTROL

3.1 Host related factors

3.1.1 *Genetic Resistance in Hosts*

The genetic make-up of any plant is a key factor in the occurrence of plant disease epidemics. Symptoms will only develop if the plants are genetically predisposed to be susceptible to a disease (Day & Wolfe, 1987). Should a specific cultivar of a host plant carry vertical resistance, the pathogen will not be able to establish it self in the plant and an epidemic will not develop. In the case of horizontal resistance of a host, some of the plants will get infected, but the epidemic will progress much slower than with a susceptible host (Agrios, 1997b).

Cultivar rotation can thus be used to supplement biological control of soilborne pathogens. In California a cultivar rotation system for cotton was developed in fields infested with *Verticillium dahliae*. This system lowered the population density of the microsclerotia in the soil, maximized yields, and permitted continuous cropping to cotton. The cultivar, Acala SJ-4, with a tolerance to *Verticillium* but a lower cotton yield, resulted in a pathogen population density below the threshold level, thus permitting the susceptible but high yielding Acala SJ-2 to be cultivated at regular intervals (Cook and Baker, 1989c).

3.2 Environment related factors

Environmental effects, also referred to as “the abiotic environment” (Cook & Baker, 1989a) present one leg of the disease triangle and have a huge

influence on disease development. These factors are also indirectly involved in the biological control of plant diseases.

3.2.1 **Soil Type**

During a field study in Oregon (USA) it was found that the beneficial effect of soil solarization differed according to soil type. In sandy soil, *Agrobacterium* spp. populations were eliminated after six weeks of soil solarization, whereas the *Agrobacterium* spp. was still detectable in a silty-clay soil after eight weeks of soil solarization (Pinkerton *et al*, 2000). Raio, Zoina & Moore (1997), obtained the same result when *A. tumefaciens* was eliminated from the sandy soil plots after a month of soil solarization, but was only significantly reduced in the silty-clay soil after two months treatment. Soil solarization trials on several North Florida soils indicated that soil colour, proportion of sand, silt and clay content as well as soil moisture were all factors affecting the temperature accumulating in the soil (Coelho, Chellemi, & Mitchell, 1999). Various research results also demonstrate that in a variety of soils, suppressiveness to certain soilborne pathogens is frequently induced by soil solarization. (Greenberger, Yogevev, & Katan, 1987; Hardy & Sivasithamparam, 1985)

Fungistasis is the phenomenon where soils are naturally suppressive to soilborne fungi due to the presence of one or more compounds that inhibit the germination of the fungi (Agrios, 1997a). These fungistatics can be volatile compounds, which can be found in all soil types. The mere presence of these compounds will, however, not ensure the suppressiveness of the soil. Certain properties of the soil can have a huge influence on the activity of these volatiles

(Romine & Baker, 1973). The activities of the volatiles are greater in alkaline than in acid soils and their effect may be nullified by certain nutrients. These substances can also be absorbed by activated charcoal in the soil (Pavlica *et al.*, 1978). Volatiles involved in biofumigation will also be subjected to all these soil properties that will have an influence on the efficacy thereof. The balance between the volatile and other biotic and abiotic factors in soil, under various environmental conditions is very important when determining the effectiveness of a volatile in suppression of a pathogen (Lewis & Papavizas, 1974).

Several soilborne diseases develop well and cause severe damage in some soils known as conducive soils, whereas they develop less and cause much milder diseases in soils known as suppressive soils. The cause of this phenomenon is not clear, but may involve biotic and/or abiotic factors and may also vary with the pathogen (Agrios, 1997b).

3.2.2 **Climate**

Successful soil solarization is climate dependant, and it's use therefore restricted to only some geographical sites. Midsummer temperatures in southeastern Australia (Porter & Merriman, 1985) as well as climatic conditions in western Australia (Hardy & Sivasithamparam, 1985) have the potential for controlling soilborne pathogens. In the USA, soil solarization was successful to reduce soil pathogen populations in California (Porter & Merriman, 1985) and western Oregon (Pinkerton *et al.*, 2000) with its high maximum day temperatures and little cloud cover. The arid climate of Israel is adequate for soil solarization to successfully control root diseases (Porter & Merriman, 1985). In Europe, soil solarization controlled various soilborne diseases in the south of

Spain (Lopez-Escudero & Blanco-Lopez, 2001; Lopez-Herrera *et al.*, 1997 & 1998; Melero-Vara *et al.*, 1995)

3.3 Pathogen related factors

3.3.1 *Survival and Longevity*

Knowledge of heat resistance of plant pathogens and their antagonists are essential in order to use and predict solar heating as a control measure for soilborne pathogens (Bollen, 1985; Katan, 1985). Bollen (1985) found the thermal death point (TDP) of the obligate pathogens, *Olpidium brassicae* and *Plasmodiophora brassicae*, above 60°C and 55°C, respectively. Although *Synchytrium endobioticum* survived 55°C, it lost its virulence. Oomycetes are heat sensitive fungi, with *Pythium aphanidermatum* the most resistant specie. *Fusarium* spp. (non-obligate pathogens) are amongst the most heat resistant pathogens with *F. oxysporum* surviving 60°C. A treatment of 70°C eliminated all saprophytes, including *Trichoderma* spp. The non-defoliating pathotype of *Verticillium dahliae* on potatoes, is more temperature-sensitive than the defoliating pathotype on cotton (Melero-Vara *et al.*, 1995)

There is a difference in sensitivity of pathogenic fungi to fungicidal isothiocyanates (ITC's) released by *Brassica* green manures, (Kirkegaard, Wong & Desmarchelier, 1996; Sarwar, & Kirkegaard, 1998; Sarwar, Kirkegaard & Wong, 1998). *In vitro* studies with five different soilborne fungal pathogens showed a definite trend where *Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia solani* were most sensitive to ITC's, *Fusarium graminearum* was intermediate and *Bipolaris sorokiniana* and *Pythium irregulare* were generally resistant. Although the exact mechanism for this phenomenon is not know, the

authors (Kirkegaard, Wong & Desmarchelier, 1996; Sarwar *et al.*, 1998) speculated that because *Pythium* is member of the Oomycota, its cell wall composition and membrane structure differs from those of the other fungi. It is thus possible that the membrane structure may reduce the efficiency of penetration of ITC's into the cells.

3.3.2 ***Virulence of inoculum***

Soilborne pathogens with a high virulent are capable of infecting the host plant faster, thus ensuring rapid production of large quantities of inoculum (Agrios, 1997a). Exudates from the seeds of the host plants play a very important role in the infection of the seedlings of the host plants. The virulence of a soilborne pathogen in the rhizosphere or on the rhizoplane can be increased when utilizing these external nutrients / stimuli exuded by the host (Hornby, 1990; Prinsloo, 1991).

3.3.3 ***Inoculum density***

Inoculum density of the soil before soil solarization will determine disease incidence after soil solarization (Lopez-Escudero & Blanco-Lopez, 2001). Therefore soil solarization applied to soil with an excessively high inoculum pressure, like in the case of artificial inoculation, will not result in a significant reduction of the disease incidence. (Hardy & Sivasithamparam, 1985). The inoculum pressure will also determine the duration and success of a crop rotation system.

4.0 CONCLUSIONS

As mentioned in this literature study, chemical control - especially soil fumigation - remains the most effective way to reduce the effects of soilborne pathogens on crop production in agriculture. However, the phase-out of well-known products like methyl bromide, ethylene di-bromide and eventually all environmentally harsh chemicals, is a reality and on the agenda of all countries legislation policies. Combining biological control tactics can become the only tool in integrated control management for soilborne diseases. The biggest challenge will be to determine the most feasible sequence of applications for optimal control of each pathogen, farming system and geographic area. We also need to stop working towards absolute terms that imply a goal of zero disease – instead of soilborne disease **control**, we must work towards soilborne disease **management**.

5.0 LITERATURE CITED

- Abid, M., and Maqbool, M.A. 1990. Effects of inter-cropping of *Tagetes erecta* on root-knot disease and growth of tomato. International Nematology Network Newsletter (Pakistan). 7: 41-42. (Abstr.).
- Agrios, N. G. 1997a. Parasitism and Disease development. Pages 43-62. In: Plant Pathology, Fourth Edition. (Ed. Agrios N G) Academic Press, London, UK.
- Agrios, N. G. 1997b. Plant Disease Epidemiology. Pages 153-172. In: Plant Pathology, Fourth Edition. (Ed. Agrios N G) Academic Press, London, UK.
- Agrios, N. G. 1997c. Control of Plant Diseases. Pages 173-221. In: Plant Pathology, Fourth Edition. (Ed. Agrios N G) Academic Press, London, UK.
- Ahmad, Y., Hameed, A. and Aslam, M. 1996. Effect of soil solarization on corn stalk rot. Plant and Soil 179:17-24.
- Akiew, S., Trevorrow, P. R., and Kirkegaard, J. 1996. Mustard green manure reduces bacterial wilt. ACIAR: Bacterial wilt Newsletter no 13:5-6.
- Alexander, S.A., and Waldenmaier, C.M. 2002. Suppression of *Pratylenchus penetrans* population in potato and tomato using African Marigold. Journal of Nematology 34(2): 130-134.
- Angus, J. F., Gardner, P. A., Kirkegaard, J. A., and Desmarchelier, J. M. 1994. Biofumigation: Isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. Plant and Soil 162:107-112.

- Antoniou, P. P., Tjamos, E. C., and Panagopoulos, C. G. 1995. Use of soil solarization for controlling bacterial canker of tomato in plastic houses in Greece. *Plant Pathology* 44:438-447.
- Arthy, J.R., Akiew, E.B., Kirkegaard, J.A., and Trevorrow, P.R. 2002. Using *Brassica* spp. as biofumigants to reduce the population of *Ralstonia solanacearum*. In: Proceedings of the 3rd International Bacterial Wilt Symposium. Pg 25. (Eds. Prior, P., Allen C. & Elphinstone, J.)
- Baker, K.F., and Cook, R.J. 1982. Biological Control in Plant Pathology. In: Biological Control of Plant Pathogens. Chapter 3. Pages 48-60. The American Phytopathological Society, Minnesota, USA.
- Bendavid-Val, R., Rabinowch, H. D., Katan, J., and Kapulnik, Y. 1997. Viability of VA-mycorrhizal fungi following soil solarization and fumigation. *Plant and soil* 195:185-193.
- Bollen, G. J. 1985. Lethal temperatures of soil. Pages 191-193. In: Ecology and Management of Soilborne Plant Pathogens: Proceedings of Section 5 of the International Congress of Plant Pathology. (Eds. Parker, C. A., Rovira, A. D., Moore, K. J., Wong, P. T. W., and Kollmorgen, J. F.). The American Phytopathological Society, Minnesota, USA.
- Campbell, R., and Greaves, M.P. 1990. Anatomy and Community Structure of the Rhizosphere. Chapter 2, Pages 11 -34. In: The Rhizosphere. (Ed. Lynch, J.M.) John Wiley & Sons.

- Castro, A.A.E., Zavaleta-Meji, A.E., Cid del Pardo, V.I., and Zamudio, G.V. 1990. Crop rotation and incorporation into the soil of *Tagetes erecta* residues for the management of *Meloidogyne incognita* (Kofoid & White) Chitwood in tomato (*Lycopersicon esculentum* Mill.) at Tecamachalco, Puebla. (Abstr.) *Revista Mexicana de Fitopatología* 8:173-180.
- Caswell, E.P., Tang, C.S., Apt, W.J. and De Frank, J. 1991. The influence of root exudates of *Chloris gayana* and *Tagetes patula* on *Rotylenchulus reniformis*. (Abstr.) *Revue de Nematologie* 14:581-587.
- Chase, C. A., Sinclair, T. R., and Locascio, S. J. 1999. Effects of soil temperature and tuber depth on *Cyperus* spp. control. *Weed Science* 47:467-472.
- Chellemi, D. O., and Olson, S. M. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in Northern Florida. *Plant Disease* 78:1167-1172.
- Chellemi, D. O., Olson, S. M., Mitchell, D. J., Secker, I., and McSorley, R. 1997. Adaptation of soil solarization to the integrated management of soilborne pests of tomato under humid conditions. *Phytopathology* 87:250-258.
- Coelho, L., Chellemi, D. O., and Mitchell, D. J. 1999. Efficacy of solarization and cabbage amendment for the control of *Phytophthora* spp. in North Florida. *Plant Disease* 83:293-299.
- Cook, R. J., and Baker, K. F. 1989a. Why Biological Control. Chapter 1. Page 1-29. In: *The Nature and Practice of Plant Pathogens*. APS Press, Minnesota, USA.

- Cook, R. J., and Baker, K. F. 1989 b. Components of Biological Control. Chapter 3. Page 57-83. In: The Nature and Practice of Plant Pathogens. APS Press, Minnesota, USA.
- Cook, R. J., and Baker, K. F. 1989c. Agricultural Practices and Biological Control. Chapter 11. Page 391-425. In: The Nature and Practice of Plant Pathogens. APS Press, Minnesota, USA.
- Croes, A.F., van den Berg, A.J.R., Bosveld, M., Breteler, H., and Wullems, G.J. 1989. Thiophene accumulation in relation to morphology in roots of *Tagetes patula*. *Planta* 179:43-50.
- Day, P. R., and Wolfe, M. S. 1987. The genetic basis of epidemics. Pages 3-6. In: Populations of Plant Pathogens. Their Dynamics and genetics. (Eds. Wolfe, M. S., and Caten, C. E.). Blackwell Scientific Publications.
- De Villiers, D. A, 1987. Invloed van wisselbougewasse op die swartstamswam (*Phytophthora nicotianae* var. *nicotianae*) van tabak. *Phytophylactica* 19:115-116.
- Eddy, D. 1999. Replacing the favored fumigant. Pages 20-23. In: AVG – Pest Control.
- Fortnum, B. A., and Currin, R. E. 1993. Crop rotation and nematicide effects on the frequency of *Meloidogyne* spp. in a mixed population. *Phytopathology* 83:350-355.
- Fortnum, B. A., Lewis, S. A., and Johnson, A. W. 2001. Crop Rotation and Nematicides for Management of Mixed Populations of *Meloidogyne* spp. on tobacco. Supplement to the *Journal of Nematology*, 33:318-324.

- Gamliel, A., and Stapleton, J. J. 1995. Improved soil disinfestation by biotoxic volatile compounds generated from solarized, organic-amended soil. *Acta Horticulturae* 382:129-137.
- Greenberger, A., Yogeve, A., and Katan, J. 1987. Induced suppressiveness in solarized soils. *Phytopathology* 77:1663-1667.
- Griffin, D. M., 1985. Soil as an environment for the growth of root pathogens. Pages 187-190. In: *Ecology and Management of Soilborne Plant Pathogens: Proceedings of Section 5 of the International Congress of Plant Pathology*. (Eds. Parker, C. A., Rovira, A. D., Moore, K. J., Wong, P. T. W., and Kollmorgen, J. F.) The American Phytopathological Society, Minnesota, USA.
- Hardy, G. E. ST. J., and Sivasithamparam, K. 1985. Soil solarization: Effects on *Fusarium* wilt of Carnation and *Verticillium* wilt of Eggplant. Pages 279-281. In: *Ecology and Management of Soilborne Plant Pathogens: Proceedings of Section 5 of the International Congress of Plant Pathology*. (Eds. Parker, C. A., Rovira, A. D., Moore, K. J., Wong, P. T. W., and Kollmorgen, J. F.) The American Phytopathological Society, Minnesota, USA.
- Harman, G.E., and Lumsden, R.D. 1990. Biological Disease Control. Chapter 10. Pages 259-280. In: *The Rhizosphere*. (Ed.Lynch, J.M.) John Wiley & Sons.
- Hornby, D. 1990. Root Diseases. Chapter 9, Pages 233-258. In: *The Rhizosphere*. (Ed. Lynch, J.M.) John Wiley & Sons.

- Jacobs, J.J.M.R., Engelberts, A., Croes, A.F., and Wullems, G.J. 1994. Thiophene synthesis and distribution in young developing plants of *Tagetes patula* and *Tagetes erecta*. *Journal of Experimental Botany* 279: 1459-1466.
- Katan, J. 1985. Solar disinfestations of soils. Pages 274-278. In: *Ecology and Management of Soilborne Plant Pathogens: Proceedings of Section 5 of the International Congress of Plant Pathology*. (Eds. Parker, C.A., Rovira, A. D., Moore, K. J., Wong, P. T. W., and Kollmorgen, J. F.) The American Phytopathological Society, Minnesota, USA.
- Katan, J., Fisher, G., and Grinstein, A. 1983. Short- and long-term effects of soil solarization and crop sequence on *Fusarium* wilt and yield of cotton in Israel. *Phytopathology* 73:1215-1219.
- Katar, M., and Alam, M. M. 1992. Effect of crop residue amendments to soil for the control of plant-parasitic nematodes. *Bioresource Technology (India)*. 41:81-83. (Abstr.)
- Keinath, A. P. 1995. Reductions in inoculum density of *Rhizoctonia solani* and control of Belly rot on pickling cucumber with solarization. *Plant Disease* 79: 1213-1219.
- Kirkegaard, J. A., and Matthiessen, J.N. 1999. Biofumigation Research – Beyond Empiricism. Pages 155-157. In: *Proceedings of the First Australian Soilborne Disease Symposium*. (Ed. Magarey, R.C.) Watson Ferguson Company Ltd., Brisbane.

- Kirkegaard, J. A., and Sarwar, M. 1999. Glucosinolate profiles of Australian canola (*Brassica napus annua* L.) and Indian mustard (*Brassica juncea* L.) cultivars: implications for biofumigation. *Australian Journal of Agricultural Research* 50:315-324.
- Kirkegaard, J. A., Wong, P. T. W., and Desmarchelier. 1996. In vitro suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathology* 45:593-603.
- Kollmorgen, J. F., Griffith, J. B., and Walscott, D. M. 1983. The effect of various crops on the survival and carry-over of wheat take-all fungus *Gaeumannomyces graminis* var. *tritici*. *Plant Pathology*. 32:73-77.
- Lewis, J. A., and Papavizas, G. C. 1970. Evolution of volatile sulfur-containing compounds from decomposition of crucifers in soil. *Soil Biol. Biochem.* 2:239-246.
- Lewis, J. A., and Papavizas, G. C. 1974. Effects of volatiles from decomposing plant tissues on pigmentation, growth, and survival of *Rhizoctonia solani*. Volume 118, No 3, Pages 56-163. In: *Soil Science*. The Williams & Wilken Co.
- Lodha, S., Sharma, S. K., and Aggarwal, R. K. 1997. Solarization and natural heating of irrigated soil amended with cruciferous residues for improved control of *Macrophomina phaseolina*. *Plant Pathology* 46:86-190.
- Lopez-Escudero, F. J., and Blanco-Lopez, M. A. 2001. Effect of a single or double soil solarization to control *Verticillium* wilt in established olive orchards in Spain. *Plant Disease* 85:489-496.

- Lopez-Herrera, C. J., Perez-Jimenez, R. M., Basallote-Ureba, M. J., Zea-Bonilla, T., and Melero-Vara, J. M. 1997. Effect of soil solarization on the control of *Phytophthora* root rot of avocado. *Plant Pathology* 46:329-340.
- Lopez-Herrera, C. J., Perez-Jimenez, R. M., Zea-Bonilla, T., Basallote-Ureba, M. J., and Melero-Vara, J. M. 1998. Soil solarization in established avocado trees for control of *Dematophora necatrix*. *Plant Disease* 82:1088-1092.
- Lucas, G. B., 1975. Cropping Systems in Relation to Tobacco Diseases. Chapter 3, Pages 21-34. In: *Diseases of Tobacco*. 3rd Ed. Harold E. Parker, N.C. USA.
- Lynch, J. M., 1990. Introduction: Some Consequences of Microbial Rhizosphere Competence for Plant and Soil.. Chapter 1, Pages 1-10. In: *The Rhizosphere*. (Ed. Lynch, J.M.) John Wiley & Sons.
- Matthiessen, J.N., and Kirkegaard, A. 1998. Biofumigation for managing soil-borne pests – progress, pitfalls and prospects. Pages 364-372. In: *Pest Management – Future Challenges*. Proceedings of the 6th Australasian Applied Entomological Research Conference Brisbane, Australia. (Eds. Zalucki, M.P., Drew, R.A.I., and White, G.G.) The University of Queensland Printery.
- Matthiessen, J.N., and Kirkegaard, A. 2006. Biofumigation and enhanced biodegradation: Opportunity and challenge in soilborne pest and disease management. *Critical Reviews in Plant Sciences* 25:235-265.
- Mayton, H. S., Olivier, C., Vaughn, S. F., and Loria, R. 1996. Correlation of fungicidal activity of *Brassica* species with Allyl isothiocyanate production in macerated leaf tissue. *Phytopathology* 86:267-271.

- McGovern, R. J., McSorley, R., and Urs, R. R. 2000. Reduction of *Phytophthora* blight of Madagascar periwinkle in Florida by soil solarization in autumn. *Plant Disease* 84:185-191.
- Melero-Vara, J. M., Blanco-Lopez, M. A., Bejarano-Alcazar, J., and Jimenez-Diaz, R. M., 1995. Control of *Verticillium* wilt of cotton by means of soil solarization and tolerant cultivars in southern Spain. *Plant Pathology* 44:250-260.
- Mojtahedi, H., Santo, G.S., Hang, A.N., and Wilson, J.H. 1991. Suppression of root-knot nematode population with selected rapeseed cultivars as green manure. *Journal of Nematology* 23: 170-174.
- Mojtahedi, H., Santo, G.S., Wilson, J.H. and Hang, A.N. 1993. Managing *Meloidogyne chitwoodi* on potato with rapeseed as green manure. *Plant Disease* 77: 42-46.
- Pavlica, D. A., Hora, T. S., Bradshaw, J. J., Skogerboe, R. K., and Baker, R. 1978. Volatiles from soil influencing activities of soil fungi. *Phytopathology* 68: 758-765.
- Pinkerton, J. N., Ivors, K. L., Miller, M. L., and Moore, L. W. 2000. Effect of soil solarization and cover crops on populations of selected soilborne plant pathogens in western Oregon. *Plant Disease* 84: 952-960.
- Plant Disease Management Strategies. 2003.
<http://www.aspnnet.org/education/AdvancedPlantPath/Topics/Epide...>
- Ploeg, A.T. 2002. Effects of selected marigold varieties on root-rot nematodes and tomato and melon yields. *Plant Disease* 86: 505-508.

- Porter, I. J., and Merriman, P. R. 1985. Evaluation of soil solarization for control of clubroot of Crucifers and white rot of Onions in southeastern Australia. Pages 282-284. In: Ecology and Management of Soilborne Plant Pathogens: Proceedings of Section 5 of the International Congress of Plant Pathology. (Eds. Parker, C. A., Rovira, A. D., Moore, K. J., Wong, P. T. W., and Kollmorgen, J. F.) The American Phytopathological Society, Minnesota, USA.
- Prinsloo, G.C. 1991. The aetiology and control of soilborne chicory diseases in the Eastern Cape. PhD. (Plant pathology) Thesis. University of the Free State.
- Raio, A., Zoina, A., and Moore, L. W. 1997. The effect of solar heating of soil on natural and inoculated agrobacteria. *Plant Pathology* 46:320-328.
- Ramirez-Villapudua, J., and Munnecke, D. E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. *Phytopathology* 78:289- 295.
- Reid, C.P.P. 1990. Mycorrhizas. Chapter 11. Pages 281-315. In: The Rhizosphere. (Ed. Lynch, J.M.) John Wiley & Sons.
- Reynolds, L.B., Potter, J.W., and Ball-Coelho, B.R. 2000. Crop rotation with *Tagetes* sp. is an alternative to chemical fumigation for control of root-lesion nematodes. *Agronomy Journal* 92(5): 957-966
- Romine, M., and Baker, R., 1973. Soil fungistasis: evidence for an inhibitory factor. *Phytopathology* 63:756-759.

- Rovira, A. D., and Venn, N. R. 1985. Effect of rotation and tillage on take-all and *Rhizoctonia* root rot in wheat. Pages 255-258. In: Ecology and Management of Soilborne Plant Pathogens: Proceedings of Section 5 of the International Congress of Plant Pathology. (Eds. Parker, C. A., Rovira, A. D., Moore, K. J., Wong, P. T. W., and Kollmorgen, J. F.) The American Phytopathological Society, Minnesota, USA.
- Sang, J. P., Minchinton, I. R., Johnstone, P. K., and Truscott, R. J. W. 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Canadian Journal of Plant Science 64:77-93.
- Sarwar, M., and Kirkegaard, J.A. 1998. Biofumigation potential of brassicas 11. Effect of environment and ontogeny on glucosinolate production and implications for screening Plant and Soil 201: 91-101.
- Sarwar, M., Kirkegaard, J.A., Wong, P.T., and Desmarchelier, J.M. 1998. Biofumigation potential of brassicas 111. In vitro toxicity of isothiocyanates to soilborne fungal pathogens. Plant and Soil 201:103-112.
- Schepman, M.A., and Jansen, M. 1994. *Tagetes patula* for control of root nematodes. Fruitteelt (Den Haag) 84: 14-15 (Abst.).
- Stapleton, J. J., and De Vay, J. E. 1982. Effect of soil solarization on populations of selected soilborne microorganisms and growth of deciduous fruit tree seedlings. Phytopathology 72:323-326.
- Stapleton, J. J., and De Vay, J. E. 1983. Response of phytoparasitic and free-living nematodes to soil solarization and 1,3-Dichloropropene in California. Phytopathology 73:1429-1436.

- Stapleton, J. J., and De Vay, J. E. 1984. Thermal components of soil solarization as related to changes in soil and root micro flora and increased plant growth response. *Phytopathology* 74:255-259.
- Tang, W., and Wat, C.K., and Towers, G.H.N. 1987. Thiophenes and benzofurans in the undisturbed rhizosphere of *Tagetes patula* L. *Plant and Soil* 98: 93-97.
- Terblanche, J. and de Villiers, D.A. 1997. The suppression of *Ralstonia solanacearum* by Marigolds. Pages 325-331. In: *Bacterial Wilt: Molecular and Ecological Aspects* (Eds. Prior, P. Allen, C. and Elphinstone, J.) INRA Editions, Paris and Springer Germany.

Table 1. Reported soilborne pathogens controlled by soil solarization

Pathogen	Reference
<i>Agrobacterium rhizogenes</i>	Pinkerton <i>et al.</i> , 2000
<i>Clavibacter michiganensis</i>	Antoniou <i>et al.</i> , 1995
<i>Dermatophora necatrix</i>	Lopez-Herrera <i>et al.</i> , 1998
<i>Fusarium</i> spp.	Chellemi & Olson, 1994 Ramirez-Vallapudua & Munnecke, 1988) Katan <i>et al.</i> , 1983
<i>Macrophomina phaseolina</i>	Ahmad, Hameed & Aslam, 1996 Lodha, Sharma & Aggarwal, 1997
<i>Meloidogyne</i> spp.	Chellemi <i>et al.</i> , 1997
<i>Paratrichodorus minor</i>	Chellemi <i>et al.</i> , 1997
<i>Pratylenchus penetrans</i>	Pinkerton <i>et al.</i> , 2000
<i>Phytophthora</i> spp.	Chellemi & Olson, 1994 Lopez-Herrera <i>et al.</i> , 1997 McGovern <i>et al.</i> , 2000 Pinkerton <i>et al.</i> , 2000
<i>Plasmodiophora brassicae</i>	Porter & Merriman, 1985
<i>Pythium</i> spp.	Gamliel & Stapelton, 1995 Stapleton & De Vay, 1984
<i>Rhizoctonia</i> sp.	Keinath, 1995 Lewis & Papavizas, 1974
<i>Sclerotium cepivorum</i>	Porter & Merriman, 1985
<i>Sclerotium rolfsii</i>	Katan <i>et al.</i> , 1983
<i>Thielaviopsis basicola</i>	Keinath, 1995
<i>Verticillium dahliae</i>	Lopez-Escudero & Blanco-Lopez, 2001 Pinkerton <i>et al.</i> , 2000) Melero-Vara <i>et al.</i> , 1995.

Table 2. Reported soilborne pathogens controlled by biofumigation and biofumigation in combination with solarization.

Pathogen	Reference
Biofumigation with thiocyanates	
<i>Aphanomyces euteiches</i>	Lewis & Papavizas, 1970
<i>Bipolaris sorokiniana</i>	Kirkegaard, Wong & Desmarchelier, 1996
<i>Fusarium graminearum</i>	Kirkegaard, Wong & Desmarchelier, 1996 Sarwar <i>et al.</i> , 1998
<i>Fusarium sambucinum</i>	Mayton, <i>et al.</i> , 1996
<i>Gaeumannomyces graminis</i> var. <i>tritici</i> ,	Kirkegaard, Wong & Desmarchelier, 1996 Sarwar <i>et al.</i> , 1998 Angus <i>et al.</i> , 1994
<i>Meloidogyne chitwoodi</i> (race 1 & 2)	Mojtahedi <i>et al.</i> , 1991; 1993
<i>Pythium irregulare</i>	Kirkegaard, Wong & Desmarchelier, 1996 Sarwar <i>et al.</i> , 1998
<i>Ralstonia solanacearum</i>	Akiew, Trevorrow & Kirkegaard, 1996 Arthy <i>et al.</i> , 2002
<i>Rhizoctonia solani</i>	Kirkegaard, Wong & Desmarchelier, 1996 Sarwar <i>et al.</i> , 1998
Biofumigation in combination with solarization	
<i>Fusarium oxysporum</i>	Ramires-Villapudua & Munnecke, 1988
<i>Pythium ultimum</i>	Gamliel & Stapleton, 1995
<i>Verticillium dahliae</i>	Gamliel & Stapleton, 1995
Biofumigation with thiophenes	
<i>Ralstonia solanacearum</i>	Terblanche & de Villiers, 1997
<i>Pratylenchus penetrans</i>	Alexander & Waldenmaier, 2002 Schepman & Jansen, 1994
<i>Meloidogyne javanica</i>	Abid & Maqbool, 1990
<i>Meloidogyne incognita</i>	Katar & Alam. 1992 Castro <i>et al.</i> , 1990 Ploeg, 2001
<i>Rotylenchulus reniformis</i>	Caswell <i>et al.</i> , 1991

EVALUATION OF CROPS FOR RHIZOSPHERE SUPPRESSION OF

Ralstonia solanacearum

ABSTRACT

The purpose of this study was to evaluate possible rotational crops for rhizosphere suppression of *Ralstonia solanacearum* (Race 1, Biovar III) the cause of bacterial wilt. A series of summer- and winter crops were evaluated in the greenhouse for rhizosphere suppression of *R. solanacearum*. The test crops were cultivated in soil with a predetermined, high population of the pathogen. After two to four months of growth, the pathogen populations present in the rhizospheres of each crop were quantified, and statistically compared with each other, as well as with the initial pathogen population of the soil. The results proved that *Tagetes spp.* (marigolds) repeatedly reduced the pathogen population significantly. *Tagetes patula* reduced the pathogen count below the infection threshold, even when planted in the same container with susceptible tobacco plants. Onions and garlic proved to be symptomless carriers or latent hosts under unfavourable winter conditions.

INTRODUCTION

Just over a century has passed since the plant pathogen *Ralstonia solanacearum* E.F. Smith, the cause of bacterial wilt of more than 500 plant species, was isolated and described for the first time by Erwin F. Smith in 1896. In years to follow, this soilborne disease was reported on every continent and most islands (Kelman, Hartman & Hayward, 1994). The destructiveness of this pathogen and its exceptional ability to survive in soil (Hayward, 1986), plant debris and the roots of latent hosts (Graham, Jones, & Lloyd, 1979; Granada & Sequeira, 1983) as well as its broad host spectrum, contribute to massive crop losses (Kelman, 1998).

Control of bacterial wilt has so far only been moderately effective, and is based on host resistance, chemical control and crop rotation. Due to some biotic- and abiotic factors, breeding for host resistance is not successful for all host species, (Hayward, 1986). The use of fumigant formulations that consist of EDB and chloropicrin mixtures are partially successful for disease control (Melton, 1991). These products are, however, unpleasant to handle and also extremely expensive. Crop rotation with non-host crops is the preferred control strategy worldwide for bacterial wilt caused by *R. solanacearum* (Akiew & Trevorrow, 1994; Hayward, 1991; Melton, 1991).

The extended host range, which also includes latent or symptomless hosts of *R. solanacearum*, makes it difficult to find suitable, rotational crops for the control of bacterial wilt (Hayward, 1986). In 1983, Granada and Sequeira described the survival of *R. solanacearum* and its isolation from the rhizosphere of plant roots as a test for screening likely rotational crops. It was also found that the pathogen population showed a definite decline in the rhizosphere of

several resistant hosts and presumed non-hosts. The purpose of the present study was to evaluate crops for rhizosphere suppression, and also for latent host status of *R. solanacearum* race1 (Buddenhagen, Sequeira & Kelman, 1962), biovar III (Hayward, 1964) in the greenhouse.

MATERIALS AND METHODS

Greenhouse Trials

Soil Preparation. Soil (88% sand, 4% silt and 8% clay), naturally infested with *R. solanacearum* (race 1, biovar III), was used in all trials. The natural pathogen population of the soil was determined, and then artificially increased to about 1×10^7 colony-forming units per gram oven-dried soil (cfu/g). A tetrazolium based, semi-selective medium, Special Medium South Africa (SMSA), (Engelbrecht, 1994) was used to quantify the *R. solanacearum* population in the soil (Fig. 1). The pathogen population in the soil was then artificially increased by mixing a suspension of *R. solanacearum*, with the soil. The pathogen suspension was standardized with a single beam spectrophotometer. At a wavelength of 600nm, a water suspension of *R. solanacearum*, with an optical density of 0,44, contains 1×10^9 cfu/ml. After the cell suspension was mixed with soil, the actual number of cells/unit present in the soil was once again determined. Pots, 2l, were filled with the infested soil and watered to field capacity.

First summer crop trial. The first group of summer crops evaluated for rhizosphere suppression of *R. solanacearum* were *Sorghum* sp. (pasture sorghum, cv. Silk); *Glycine max* (L.) Merr. (sojabeans, cv. Forrest); *Gossypium hirsutum* L. (cotton, cv's. Sicala & Acala 1517/70); *Eragrotis tef* (Zucc.) Trotter.

(teff, cv. SA Brown) and *Tagetes patula* (dwarf marigolds). As controls, *Nicotiana tabacum* L. (tobacco, the susceptible cv. TL33) and a bare fallow pot, filled with the infested soil, were included. To evaluate the influence of intercropping, a possible suppressing non-host and a highly susceptible host on the pathogen population of the soil, marigolds and tobacco (cv. TL33) respectively, were planted in the same pot and included as a treatment. The crops were grown for two months at 30°C and a relative humidity of 80%.

Second summer crop trial. During this trial, *Tagetes minuta* L. (wild marigold or Khaki-bush), *Chrysanthemum cinerariifolium* (pyrethrum) and again *T. patula*, were tested for rhizosphere suppression of *R. solanacearum*. The tobacco cultivar, TL33, and a bare fallow pot, again served as controls. These crops were also grown for two months at 30°C and a relative humidity of 80%.

Third summer crop trial. This trial consisted of herbaceous summer crops, *Sinapis alba* (mustard), *Allium tuberosum* (spring onions), *Ocimum basilicum* (sweet basil), *Coriandrum sativa* L. (coriander) as well as *Tagetes erecta* (giant marigold) and *T. patula*. Tobacco (cv TL33) was used as a control. The plants were grown for two months in the greenhouse at 30°C and a relative humidity of 80%.

Winter crop trial. Winter crops evaluated for rhizosphere suppression were *Allium sativum* L. (garlic), *Allium cepa* L. (onions) and *Triticum aestivum* L. (wheat). Tobacco was not included as a control because of the low soil temperature ($\pm 16^{\circ}\text{C}$) at which this trial was conducted. The bare fallow pot served as a standard. Due to the nature of these crops, they were grown for four months at $\pm 16^{\circ}\text{C}$.

At the end of each trial, the rhizosphere population of *R. solanacearum* was determined for each crop. Rhizosphere soil was suspended in de-ionized, sterile water to a ratio of 1:10 (m/v) and shaken on a rotary shaker for 30 minutes (Granada & Sequeira, 1983). Different dilutions of this suspension were plated on the SMSA semi-selective medium, and incubated at 30°C. After five days incubation, typical colonies were counted and the pathogen population of each crop calculated.

Statistical analyses. All trials were laid out as randomized complete block designs with four replications (four replications). A \log^{10} transformation was done on the values of the soil's colony counts and Bonferroni's multiple comparison test was used to determine significant differences at the 5% test level. Colony counts of the different test crops and controls were compared with each other, but also with the initial pathogen population in the soil.

RESULTS

Summer Crops

First trial. After two months growth in the *R. solanacearum* infected soil, the pathogen population in the rhizosphere of the marigolds was the lowest, and differed significantly from six of the nine other treatments (Table 1). Only the marigolds and marigold/tobacco mixture gave a significantly lower rhizosphere count than both the controls. Tobacco plants in the control pots were all dead or severely wilted, whereas tobacco intercropped with marigolds did not show any symptoms. The rhizosphere pathogen populations of the other test crops did not differ significantly from each other.

Second trial. The results of the second trial confirmed the favourable reduction that marigold roots have on the pathogen. After two months the population of *R. solanacearum* in the rhizosphere of *T. patula* was $3,6 \times 10^3$ times lower than that of the initial pathogen population of $4,7 \times 10^6$ cfu/g in the soil (Table 2). The rhizosphere pathogen count of *T. patula* was not only significantly lower than the initial count, but was also lower than that of all the test crops as well as the two controls. Of the other test crops only pyrethrum had a rhizosphere count that differed significantly from the initial population of the soil (Table 2).

Third trial. Of all the herbaceous crops, only the rhizosphere pathogen populations of coriander and the two-marigold *spp.* were significantly lower than that of the tobacco control (Table 3).

Winter Crops

After four months cultivation at $\pm 16^\circ\text{C}$, the pathogen population in the rhizospheres of garlic and onions still prevailed at 10^6 cfu/g soil (Table 4). Although no symptoms were expressed, the pathogen was also isolated from inside the bulbs of both these two species. The rhizosphere population of the wheat was significantly lower than the initial pathogen population in the soil.

DISCUSSION

In the present study *T. patula* and *T. erecta* significantly reduced the rhizosphere population of *R. solanacearum*. Even when intercropped with the highly susceptible tobacco cultivar, TL33, they reduced the pathogen population below the infection threshold, and consequently no symptoms developed on the

tobacco plants. Wheat was recognized as a favourable winter rotation crop for the suppression of *R. solanacearum*, whereas onions and garlic were indicated as latent hosts by sustaining a high pathogen count in their rhizospheres under winter conditions.

To our knowledge, this is the first report of the ability of *Tagetes spp.* to suppress *R. solanacearum*. The inhibiting effect that *Tagetes spp.* has on nematodes is widely known and published (Schepman & Jansen, 1994; Ploeg, 2002). According to Caswell *et al.* (1991) root secretions from the undisturbed rhizosphere of *T. patula* are toxic to the reniform nematode. The root secretions of *T. patula* and *T. erecta* that cause the suppression of *R. solanacearum* will have to be investigated and confirmed. Thiophene, a heterocyclic, sulphurous compound with strong biocidal activity, can be extracted from the undisturbed rhizosphere of *T. patula* and *T. erecta* (Croes *et al.*, 1989; Jacobs *et al.*, 1994, and Tang, Wat & Towers, 1987). This natural biocide, with a higher concentration in the roots than in the rest of the plant (Jacobs *et al.*, 1994), might thus be responsible for the suppression of *R. solanacearum* in the rhizosphere of *T. patula* and *T. erecta*

In the present study inter-cropping *T. patula* and susceptible tobacco plants in infected soil resulted in the susceptible tobacco being protected from infestation, due to sufficient reduction of the pathogen population in the soil. Sufficient toxic secretions of the *T. patula*'s root system presumably caused the significant reduction of the pathogen population in both rhizospheres. Abid & Maqbool (1990) also found that the galls caused by *M. javanica* on the roots of tomatoes, were significantly fewer when the tomatoes were inter-cropped with *T. erecta*. Both Castro *et al.* (1990) and Akhtar & Alam (1992) reported that

crop rotation with *T. erecta* and the incorporation of *T. erecta* in the soil, not only caused a reduction of the *M. incognita* population, but also resulted in a reduced gall index and increased tomato- and chili yields. Further research is now essential to determine whether this suppression of *R. solanacearum* by *Tagetes. spp.*, observed in the greenhouse, can be used as a biological control agent for bacterial wilt under commercial farming practices.

Onions and garlic are not listed as hosts to *R. solanacearum*. These two winter crops, however, supported the survival of *R. solanacearum* (race 1, biovar III) in their rhizospheres under winter conditions that are unfavourable to this pathogen. According to N Mienie at ARC-VOPI (Personal communication) onions also tested positive as a symptomless host of *R. solanacearum* (race 3, biovar II). The isolation of the pathogen from the bulbs of both these symptomless hosts is consistent with findings that apparent non-hosts can maintain the bacterium through limited invasion and population expansion within the roots (Granada and Sequeira, 1983). Symptomless latent hosts should therefore not be planted as rotation crops on fields infested with *R. solanacearum*.

LITERATURE CITED

- Akiew, E., and Trevorrow, P.R. 1994. Management of Bacterial Wilt of Tobacco. . Pages 179-198. In: Bacterial Wilt: The Disease and its Causative Agent, *Pseudomonas solanacearum*. (Eds. Hayward, A. C., and Hartman, G. L.) CAB International, Wallingford, UK.
- Abid, M., and Maqbool, M. A. 1990. Effects of inter-cropping of *Tagetes erecta* on root-knot disease and growth of tomato. (Abstr.) International Nematology Network Newsletter (Pakistan). 7:41-42.
- Akhtar, M., and Alam, M. M. 1992. Effect of crop residue amendments to soil for the control of plant-parasitic nematodes. (Abstr.) Bioresource Technology (India). 41:81-83.
- Buddenhagen, I., Sequeira, L., and Kelman, A. 1962. Designation of races of *Pseudomonas solanacearum*. (Abstr.) Phytopathology 52:726.
- Castro, A. A. E., Zavaleta-Meji, A. E., Cid del Prado V. I., and Zamudio, G. V. 1990. Crop rotation and incorporation into the soil of *Tagetes erecta* residues for the management of *Meloidogyne incognita* (Kofoid & White) Chitwood in tomato (*Lycopersicon esculentum* Mill.) at Tecamachalcon, Puebla. (Abstr.) Revista Mexicana de Fitopatologia 8:173-180.
- Caswell, E. P., Tang, C. S., Apt, W. J. and De Frank, J. 1991. The influence of root exudates of *Chloris gayana* and *Tagetes patula* on *Rotylenchulus reniformis*. (Abstr.) Revue de Nematologie 14: 581-587.
- Croes, A. F., van den Berg, A. J. R., Bosveld, M., Breteler, H., and Wullems, G. J. 1989. Thiophene accumulation in relation to morphology in roots of *Tagetes patula*. Planta 179:43-50.

- Engelbrecht, M. C. 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. *Aciair Bacterial Wilt Newsletter* 10:3-5.
- Graham, J., Jones, D. A., and Lloyd, A. B. 1979. Survival of *Pseudomonas solanacearum* Race 3 in plant debris and in latently infected potato tubers. *Phytopathology* 69:1100-1103.
- Granada, G. A., and Sequeira, L. 1983. Survival of *Pseudomonas solanacearum* in soil, rhizosphere and plant roots. *Canadian Journal of Microbiology* 29:433-440.
- Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. *Journal of Applied Bacteriology* 27:365-277.
- Hayward, A. C. 1986. Bacterial wilt caused by *Pseudomonas solanacearum* in Asia and Australia: an Overview. Pages 15-24. In: *Bacterial wilt disease in Asia and the South Pacific*. (Ed. Presley G. J.) ACIAR, Canberra, Australia.
- Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathology* 29:65-87.
- Jacobs, J. J. M. R., Engelberts, A., Croes, A. F., and Wullems, G. J. 1994. Thiophene synthesis and distribution in young developing plants of *Tagetes patula* and *Tagetes erecta*. *Journal of Experimental Botany* 279:1459-1466.
- Kelman, A. 1998. One hundred and one years of research on bacterial wilt. Pages 1-5. In: *Bacterial Wilt: Molecular and Ecological Aspects* (Eds. Prior, P. Allen, C. and Elphinstone, J.) INRA Editions, Paris and Springer Germany.

- Kelman, A., Hartman, G. L., and Hayward, A. C. 1994. Introduction to the 1st international bacterial wilt symposium. Pages 1-7. In: Bacterial wilt: The disease and its causative agent, *Pseudomonas solanacearum*. (Eds. Hayward, A. C., and Hartman, G. L.) CAB International, Wallingford, UK.
- Melton, T. A. 1991. Effects of two-year crop rotation and cultivar resistance on bacterial wilt in flue-cured tobacco. *Plant Disease* 75:695-698.
- Ploeg, A. T. 2002. Effects of selected marigold varieties on root-knot nematodes and tomato and melon yields. *Plant Disease* 86:505-508.
- Schepman, M. A., and Jansen, M. 1994. *Tagetes patula* for control of root nematode. Kleine tuinafrikaan als bestrijder van wortellesie-aaltjie. *Fruitteelt (Den Haag)* 84:14-15 (Abstr)
- Tang, C. S., Wat, C. K., and Towers, G. H. N. 1987. Thiophenes and benzofurans in the undisturbed rhizosphere of *Tagetes patula* L. *Plant and Soil* 98:93-97.

Table 1. Rhizosphere population of *Ralstonia solanacearum* in summer crops grown for two months in infested soil^a under greenhouse conditions at 30 °C

Crop	Rhizosphere population	
	Average cfu/g soil	Average (log ₁₀) ^b
Initial pathogen population in soil	1.500 x 10 ⁶	6.176 a ^c
<i>Nicotiana tabacum</i> L. (tobacco cv. TL33/73) ^d	1.271 x 10 ⁶	6.104 a
Fallow ^e	4.581 x 10 ⁵	5.661 ab
<i>Sorghum</i> sp. (pasture sorghum cv. Silk)	3.083 x 10 ⁵	5.498 ab
<i>Glycine max</i> (L.) Merr. (soyabean cv. Forrest)	2.449 x 10 ⁵	5.389 abc
<i>Gossypium hirsutum</i> L. (cotton cv. Sicala)	1.884 x 10 ⁵	5.275 abc
<i>G. hirsutum</i> L. (cv. Acala1517/70)	1.138 x 10 ⁵	5.056 abcd
<i>Eragrostis tef</i> (Zucc) Trotter (cv. SA brown)	5.152 x 10 ⁴	4.712 bcd
<i>N. tabacum</i> & <i>Tagetes patula</i> (Dwarf marigolds)	1.901 x 10 ⁴	4.279 cd
<i>T. patula</i>	1.069 x 10 ⁴	4.029 d
LSD _{Bonferroni} (0.05)	-	1.148
SEM	-	0.225
Coefficient of variance (%)	-	8.79

^a Naturally infested soil with the pathogen population artificially increased.

^b Average log₁₀ transformed values of colony forming units of *Ralstonia solanacearum* per gram rhizosphere soil (cfu/g).

^c Values followed by the same letter do not differ significantly (P<0.05).

^d Susceptible tobacco cultivar used as a control.

^e The infested soil without a crop as a bare-fallow control

Table 2. Rhizosphere population of *Ralstonia solanacearum* in summer crops grown for two months in infested soil^a under greenhouse conditions at 30 °C

Crop	Rhizosphere population	
	Average cfu/g soil	Average (log ₁₀) ^b
Initial pathogen population in soil	4.700 x 10 ⁶	6.672 a ^c
<i>Nicotiana tabacum</i> L. (tobacco cv. TL33/73) ^d	5.984 x 10 ⁵	5.777 ab
Fallow ^e	2.275 x 10 ⁵	5.357 ab
<i>Tagetes minuta</i> L. (wild marigold / khaki bush)	1.365 x 10 ⁵	5.135 ab
<i>Chrysanthemum cinerarifolium</i> (pyrethrum)	7.379 x 10 ⁴	4.868 b
<i>Tagetes patula</i> (dwarf marigolds)	1.312 x 10 ³	3.118 c
LSD _{Bonferroni} (0.05)	-	1.676
SEM	-	0.331
Coefficient of variance (%)	-	13.9

^a Naturally infested soil with the pathogen population artificially increased.

^b Average log₁₀ transformed values of colony forming units of *Ralstonia solanacearum* per gram rhizosphere soil (cfu/g).

^c Values followed by the same letter do not differ significantly (P < 0.05)

^d Susceptible tobacco cultivar used as a control.

^e The infested soil without a crop as a bare-fallow control

Table 3. Rhizosphere population of *Ralstonia solanacearum* in herbaceous summer crops grown for two months in infested soil^a under greenhouse conditions at 30 °C.

Crop	Rhizosphere population	
	Average cfu/g soil	Average (log ₁₀) ^b
Initial pathogen population in soil	3.50 x 10 ⁷	7.544 a ^c
<i>Nicotiana tabacum</i> L. (tobacco cv. TL33/73) ^d	5.80 x 10 ⁵	5.763 b
<i>Sinapis alba</i> (white mustard)	2.64 x 10 ⁵	5.422 b
<i>Allium tuberosum</i> (chives)	1.27 x 10 ⁵	5.104 bc
<i>Ocimum basilicum</i> (sweet basil)	1.07 x 10 ⁵	5.029 bc
<i>Tagetes erecta</i> (giant marigold)	4.12 x 10 ⁴	4.525 cd
<i>T. patula</i> (dwarf marigolds)	3.35 x 10 ⁴	4.450 cd
<i>Coriandrum sativum</i> L. (coriander)	1.25 x 10 ⁴	4.097 d
LSD _{Bonferroni} (0.05)	-	0.796
SEM	-	0.152
Coefficient of variance (%)	-	5.8

^a Naturally infested soil with the pathogen population artificially increased.

^b Average log₁₀ transformed values of colony forming units of *Ralstonia solanacearum* per gram rhizosphere soil (cfu/g).

^c Values followed by the same letter do not differ significantly (P<0.05)

^d Susceptible tobacco cultivar used as a control.

Table 4. Rhizosphere population of *Ralstonia solanacearum* in winter crops grown for four months in infested soil^a under greenhouse conditions at 16 °C.

Crop	Rhizosphere population	
	Average cfu/g soil	Average (log ₁₀) ^b
Initial pathogen population of soil	1.00 x 10 ⁷	7.000 a ^c
<i>Allium sativum</i> L. (garlic)	4.57 x 10 ⁶	6.660 ab
<i>Allium cepa</i> L. (onions)	3.55 x 10 ⁶	6.550 ab
<i>Triticum aestivum</i> L. (wheat cv. Inia)	3.63 x 10 ⁵	5.560 b
Fallow ^d	1.00 x 10 ⁴	4.000 c
LSD _{Bonferroni} (0.05)	-	1.206
SEM	-	0.244
Coefficient of variance (%)	-	8.8

^a Naturally infested soil with the pathogen population artificially increased.

^b Average log₁₀ transformed values of colony forming units of *Ralstonia solanacearum* per gram rhizosphere soil (cfu/g).

^c Values followed by the same letter do not differ significantly (P<0.05).

^d The infested soil without a crop as a bare-fallow control

**MARIGOLDS AS BIOLOGICAL CONTROL AGENT OF BACTERIAL WILT
CAUSED BY
Ralstonia solanacearum
IN SOILS USED FOR TOBACCO PRODUCTION.**

ABSTRACT

Bacterial wilt, caused by *Ralstonia solanacearum* (Race 1), is an important disease of tobacco. The present study evaluated the influence of the biologically active crop, marigold (*Tagetes patula*) and other latent plant hosts as symptomless carriers on the bacterial population in the soil and subsequently on the yield of a tobacco crop. A greenhouse trial conducted in 0,5 m deep soil indicated that marigolds in combination with non-host winter crops, could reduce the pathogen population of the soil to such an extent that the disease incidence on the follow-up tobacco crop was 40% lower than that of the preceding tobacco crop. A four-year rotation trial on a heavily infested field showed that marigolds in combination with wheat were responsible for a significantly higher tobacco yield than other crop combinations. In both greenhouse and field trials cabbage (*Brassica oleracea* L. var *capitata*.) proved to be a latent host, suggesting that it should be avoided as a winter cash crop in tobacco fields infested with *R. solanacearum*.

INTRODUCTION

In South Africa control of bacterial wilt of tobacco (*Nicotiana tabacum* L.) caused by *Ralstonia solanacearum* (E F Smith) Race 1 (Buddenhagen, Sequeira & Kelman, 1962), Biovar III (Hayward, 1964) is based mainly on the planting of resistant cultivars. Crop rotation, utilizing certain pasture crops is used on large commercial farms. Farms on irrigation schemes are smaller due to the cost of land and tobacco is planted every summer followed by another cash crop during winter (Pretorius, 1996). This practice leads to a build-up of bacterial inoculum and diminished performance of tolerant tobacco cultivars (Mehan, McDonald & Subrahmanyam, 1986; Melton & Powell, 1991). There is consequently a need for non-host rotational crops that aggressively suppress *R. solanacearum* populations in the soil in a relatively short span of time.

The inhibiting effect that *Tagetes spp.* have on nematodes has been reported (Schepman & Jansen, 1994; Ploeg, 2002). According to Caswell *et al.* (1991) root secretions from the undisturbed rhizosphere of *T. patula* are toxic to the reniform nematode (*Rotylenchulus reniformis*). Both Castro *et al.* (1990) and Akhtar & Alam (1992) reported that crop rotation with *Tagetes erecta* and the incorporation of *T. erecta* in the soil not only caused a reduction in the population of *Meloidogyne incognita*, but also resulted in a reduced gall index and increased tomato and chilli yields. Abid & Maqbool (1990) also found that the galls caused by *M. javanica* on the roots of tomatoes were significantly fewer when the tomatoes were inter-cropped with *T. erecta*. Thiophene, a heterocyclic, sulphurous compound with strong biocidal activity, can be extracted from the undisturbed rhizosphere of *T. patula* and *T. erecta* (Tang, Wat & Towers, 1987; Croes *et al.*, 1989, and Jacobs *et al.* 1994). This natural

biocide is possibly responsible for the bio-fumigating properties of the *Tagetes* spp. towards the nematodes (Tang, Wat & Towers, 1987). The first objective of the present study was to determine if *Tagetes patula*. (marigolds), could be used as a bio-control agent to control bacterial populations in soil and thus increase tobacco yield.

Crop rotation systems for the control of bacterial wilt have often failed due to the inclusion of symptomless crops and weeds (Tusiime *et al.*, 1997). These plants should therefore be identified and eliminated from rotation systems. A second objective of the present study was to examine the role of latent hosts as sheltered sites for the survival of *R. solanacearum* in an integrated disease management system. According to Graham, Jones, & Lloyd, (1979) *R. solanacearum* has a poor survival ability in soil, but survives or overwinters in sheltered sites such as plant debris and the roots of latently infected alternative hosts. Symptomless plants, such as latent infected resistant chilli peppers (Abdullah & Rahman, 1997), latent infected potato tubers. (Liao *et al.*, 1997), including infected plant debris (Graham, Jones & Loyed, 1979) can harbour and lead to an increased pathogen population in the soil.

MATERIALS AND METHODS

Greenhouse Trial

Pathogen population assessment. Pasteurized, gravelly soil (88% sand, 4% silt & 8% clay) was artificially infested with *R. solanacearum* (Race 1; Biovar III). The soil was pasteurized with steam for eight hours under atmospheric pressure. The pasteurized soil was then mixed with sodium alginate beads (Bashan, 1986) containing the pathogen and placed in large

asbestos pots (0.07m³) (Fig. 2). To confirm the virulence of the pathogen, test the level of infestation and establish the pathogen in the infested soil, susceptible tobacco seedlings, (cultivar OD272), were planted in the pots. Pots were subsequently incubated in a greenhouse at an average temperature of 30 °C and relative humidity of 80%. After six weeks the pathogen population in the soil was quantified on the semi-selective SMSA medium (Engelbrecht, 1994)

Planting of winter crops. Diseased tobacco plants were removed after six weeks and the soil prepared for the planting of winter crops. Sweet potatoes (*Ipomoea batatas* L.), onions (*Allium cepa* L.) coriander (*Coriandrum sativum* L.), garlic (*Allium sativum* L.) and cabbage (*Brassica oleracea* L. var *capitata* Alef) were subsequently planted in the pots. The five treatments were laid out as a randomized complete block design with three replications. The average temperature of the greenhouse was kept at 16°C. After four months the winter crops were removed from the pots.

Planting of first tobacco crop. The chemical status of the soil was determined, and the nutritional status and pH were corrected for optimal tobacco growth (H Boshoff, ARC-IIC – Personal communication). All pots were again planted to the susceptible tobacco cultivar (OD272), and disease progress monitored to determine the influence of the winter crops on the pathogen population in the soil. The average temperature of the greenhouse was kept at 30°C and the relative humidity at 80%. The percentage of tobacco plants with bacterial wilt symptoms in each pot was recorded at four, five, seven and eight weeks after planting. After eight weeks, the plants were removed from the pots and the soil prepared for marigolds.

Planting of T. patula. Marigolds were sown in all pots as a possible bio-control agent of *R. solanacearum* (Fig. 3). The marigold plants were spaced 5 cm apart (\pm 50 plants per pot). This spacing was calculated from the recommended field density for nematode control of 5 – 7kg seed/hectare (Schepman & Jansen, 1994). The average temperature of the greenhouse was 30°C and the relative humidity 80%. After three months the marigolds were removed from the pots and the soil again prepared for tobacco cultivation.

Planting of second tobacco crop. A second crop of the susceptible tobacco cultivar was planted to test the influence of the marigolds on the pathogen population. Similar to the first planting, plants were monitored for wilt at four, five, seven and eight weeks after planting.

Field Trial

A four-year rotation trial was carried out in a field heavily infested with *R. solanacearum*. Tobacco and marigolds were planted as summer crops and cabbage and wheat (*Triticum aestivum* L.) as winter crops. The choice of crops was based on regional practices and results derived from the greenhouse trial.

Treatments. (i) A susceptible tobacco cultivar, OD272, planted during summer was followed by cabbage during winter. (ii) The susceptible tobacco cultivar in early summer was replaced by marigolds for the remainder of summer and throughout fall (these plots remained fallow during winter). (iii) Marigolds planted during summer followed by wheat during winter. All treatments were repeated for three consecutive years. During the fourth summer, a tolerant tobacco cultivar, OD2, was planted on all the plots and

cultivated under optimal conditions for tobacco production (Table 1.). The tolerant tobacco cultivar was left to grow for three months. The experiment was designed as a randomized complete block design with four replications.

Data collection and statistical analysis. The percentage survival and percentage tobacco plants with typical symptoms were determined three months after the tolerant cultivar was planted. The average plant height and green-leaf-mass of all surviving plants were measured in order to quantify the influence of the pathogen population on yield. Differences between treatments were tested in an analysis of variance (ANOVA), using the statistical program GenStat (2000).

RESULTS

Greenhouse trial pathogen population assessment. After six weeks, all tobacco plants developed typical bacterial wilt symptoms, proving that the pathogen was uniformly spread in the soil. The pathogen population in the soil was found to be above the disease threshold level of 10^5 colony-forming units per gram soil before the winter crops were planted.

Planting of first tobacco crop. Eight weeks after planting, an average of 66% of tobacco plants cultivated after the sweet potatoes developed symptoms, whereas 100% of the tobacco cultivated after the cabbage, developed symptoms and died (Table 2). Tobacco plants preceded by coriander, onion and garlic had a lower rate of disease progress than those preceded by cabbage. However, after eight weeks, these tobacco plants displayed 85-95% mortality.

Second Tobacco Planting. The rate of disease development in the tobacco plants planted after the marigolds was lower than that of plants planted directly after the winter crops (Table 2). The disease incidence in the tobacco following the sweet potatoes and marigolds was 40% lower than that of the preceding tobacco crop. This suggests a lower pathogen population in the soil following marigold cultivation.

Field Trial

Following a three-year rotation system, the performance of the tolerant tobacco cultivar provides an indication of the reduction in the pathogen population in the soil induced by the various crops and crop combinations (Table 3). In terms of survival, symptomology, plant height and green leaf mass, tobacco following the marigold/wheat combination, performed the best and differed significantly ($P < 0.05$) from treatments (i) and (ii) (Fig. 4). There were no significant differences between the other two treatments. Although significantly lower compared to the marigold/wheat plots, the mortality of tobacco in the other two treatments was less than 10%.

DISCUSSION

The present study showed that marigolds planted in combination with non-host crops such as sweet potatoes or wheat, could effectively reduce the soil population of *R. solanacearum* in pot experiments. The success of this treatment will, however, depend on initial levels of the pathogen population in the soil. In addition, it is also essential that the marigolds should be followed by a non-host, winter crop like the sweet potatoes or wheat, since a symptomless

or latent host like cabbage will maintain an infective population above the disease threshold level during winter.

In the field trial, the cultivation of marigolds in combination with wheat resulted in a 60% increase in tobacco yield (green leaf mass) compared to the control treatment where susceptible tobacco and cabbage were rotated. This result is consistent with Ploeg (2002), who found that the cultivation of marigolds on a nematode infested field resulted in a 50% increase in yield of tomatoes and melons. Despite the 60% yield loss of the tolerant tobacco on the control plots, a very low mortality rate prevailed. This suggests that cultivar tolerance was responsible for survival of the plants, but that high pathogen populations in the soil stunt the growth of the plants to such an extent that the yield is significantly reduced (Mehan, McDonald & Subrahmanyam, 1986; Melton & Powell, 1991).

The influence of a symptomless host crop on *R. solanacearum* populations was illustrated in both trials. The high mortality in the greenhouse trial, and low yield in the field trial of tobacco following the cultivation of cabbage, onions, garlic and coriander, suggests that these crops are latent or symptomless hosts of *R. solanacearum*. The long-term survival and reproduction of *R. solanacearum* in the rhizosphere and roots of symptomless hosts (Graham, Jones, & Lloyd, 1979), has significant implications for its control in tobacco fields.

In the greenhouse trial, sweet potatoes proved to be a non-host of *R. solanacearum*. This observation is inconsistent with findings of He, (1986) in China and Machmud, (1992) in Vietnam. Contrary to our results, *Tagetes spp.* have been reported as hosts of *R. solanacearum* in other geographical

localities. Jones, Robert & Benson (1996), reported that bacterial wilt, caused by *R. solanacearum*, is the most frequent disease of marigolds in the North Carolina landscapes. Weed-host studies in Uganda (Tusiime *et al.*, 1997), and Australia (Akiew & Trevorrow, 1997) also listed *T. minuta* as a host of *R. solanacearum*.

Contradictions concerning the host status of *R. solanacearum* in different geographical localities are well known. In 1994 Hayward stated that there are several anomalies in the occurrence of bacterial wilt in certain hosts in some parts of the world, but not in others where bacterial wilt is endemic. The reason for these perceived differences is not clear. The assumption was, therefore, made by Hayward (1994) that specific strains, pathogenic to these hosts, have evolved only in certain parts of the world and not in others. The fact that different cultivars, of the same crop species can differ in susceptibility towards a specific pathogen is highly relevant here.

R. solanacearum is an ubiquitous pathogen with a wide host range. No universal solutions, only principles, exist for the effective control of this highly mutable pathogen in different locations. The present study has, however, demonstrated that *Tagetes patula*, if managed as part of a rotation system with certain winter crops, will reduce the pathogen population in the soil. In so doing, the pressure on the tolerant tobacco cultivars can be significantly alleviated in order to significantly increase yield.

LITERATURE CITED

- Abdullah, H., and Rahman, A. 1997. Multiplication of *Ralstonia solanacearum* in *Capsicum annuum*. Pages 309-315. In: Bacterial Wilt: Molecular and Ecological Aspects (Eds. Prior, P. Allen, C. and Elphinstone, J.) INRA Editions, Paris and Springer Germany.
- Abid, M., and Maqbool, M. A. 1990. Effects of inter-cropping of *Tagetes erecta* on root-knot disease and growth of tomato. International Nematology Network Newsletter (Pakistan). 7:41-42. (Abstr.)
- Akhtar, M., and Alam, M. M. 1992. Effect of crop residue amendments to soil for the control of plant-parasitic nematodes. Bioresource Technology (India). 41:81-83. (Abstr.)
- Akiew, E., and Trevorrow, P. R. 1997. Management of Bacterial Wilt of Tobacco. Pages 179-198. In: Bacterial Wilt: The Disease and its Causative Agent, *Pseudomonas solanacearum*. (Eds. Hayward, A. C., and Hartman, G. L.) CAB International, Wallingford, UK.
- Bashan, Y. 1986. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Applied and Environmental Microbiology 51:1089-1098
- Buddenhagen, I., Sequeira, L., and Kelman, A. 1962. Designation of races of *Pseudomonas solanacearum*. Phytopathology 52: 726 (Abstr.).
- Castro, A. A. E., Zavaleta-Meji, A. E., Cid del Prado V. I., and Zamudio, G. V. 1990. Crop rotation and incorporation into the soil of *Tagetes erecta* residues for the management of *Meloidogyne incognita* (Kofoid & White) Chitwood in tomato (*Lycopersicon esculentum* Mill.) at Tecamachalcon, Puebla. (Abstr.) Revista Mexicana de Fitopatologia 8:173-180.

- Caswell, E. P., Tang, C. S., Apt, W. J. and De Frank, J. 1991. The influence of root exudates of *Chloris gayana* and *Tagetes patula* on *Rotylenchulus reniformis*. (Abstr.) *Revue de Nematologie* 14: 581-587.
- Croes, A. F., van den Berg, A. J. R., Bosveld, M., Breteler, H., and Wullems, G. J. 1989. Thiophene accumulation in relation to morphology in roots of *Tagetes patula*. *Planta* 179:43-50.
- Engelbrecht, M. C. 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. *Aciair Bacterial Wilt Newsletter* 10:3-5.
- GenStat for Windows. 2000. Release 4.2. Fifth Edition. VSN International Ltd., Oxford, UK.
- Graham, J., Jones, D. A., and Lloyd, A. B. 1979. Survival of *Pseudomonas solanacearum* Race 3 in plant debris and in latently infected potato tubers. *Phytopathology* 69:1100-1103.
- Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. *Journal of Applied Bacteriology*. 27: 265-277.
- Hayward, A. C. 1994. The hosts of *Pseudomonas solanacearum*. Pages 9-24. In: *Bacterial Wilt: The Disease and its Causative Agent, Pseudomonas solanacearum*. (Eds. Hayward, A. C., and Hartman, G. L.) CAB International, Wallingford, UK.
- He, L. Y. 1986. Bacterial wilt in the Peoples Republic of China. Pages 40-48. In: *Bacterial Wilt Disease in Asia and the South Pacific*. (Ed. Presley, J.) ACIAR Proceedings 13. ACIAR publications, Canberra.

- Jacobs, J. J. M. R., Engelberts, A., Croes, A. F., and Wullems, G. J. 1994. Thiophene synthesis and distribution in young developing plants of *Tagetes patula* and *Tagetes erecta*. *Journal of Experimental Botany* 279:1459-1466.
- Jones, R. K., Robert, A., and Benson, D. M. 1996. Southern bacterial wilt on marigolds. *Plant Pathology Information Notes. Ornamental Disease Information Note 9*.
- Liao, B. S., Shan, Z. N., Duan, N. X., Tan, Y. J., Lei, Y., Li, D. and Mehan, V. K. 1997. Relationship between latent infected and groundnut bacterial wilt resistance. Pages 294-299. In: *Bacterial Wilt: Molecular and Ecological Aspects* (Eds. Prior, P., Allen, C., and Elphinstone, J.) INRA Editions, Paris and Springer Germany.
- Machmud, M. 1992. Control of peanut bacterial wilt through crop rotation. Pages 221-224. In: *Bacterial Wilt*. ACIAR Proceedings No 45: (Eds. Hartman, G. L. and Hayward, A. C.) ACIAR publications, Canberra.
- Melton, T.A., and Powell, N.T. 1991. Effects of two-year crop rotation and cultivar resistance on bacterial wilt in flue-cured tobacco. *Plant Disease* 75:695-698.
- Mehan, V.K., Mc Donald, D. and Subrahmanyam, P. 1986. Bacterial wilt of groundnut: Control with emphasis on host plant resistance. In: *Bacterial Wilt Disease in Asia and the South Pacific*. Pages 112-119. ACIAR Proceedings No. 13: (Ed. Persley, G.J.) ACIAR publications, Canberra.
- Ploeg, A. T. 2002. Effects of selected marigold varieties on root-knot nematodes and tomato and melon yields. *Plant Disease* 86:505-508.

- Pretorius, L. 1996. Tuinblom beheer tabaksiekte. Landbou Weekblad (November) (970:44-45)
- Schepman, M.A., and Jansen, M. 1994. *Tagetes patula* for control of root nematodes. *Fruitteelt* (Den Haag) 84:14-15 (Abst.).
- Tang, C. S., Wat, C. K., and Towers, G. H. N. 1987. Thiophenes and benzofurans in the undisturbed rhizosphere of *Tagetes patula* L. *Plant and Soil* 98:93-97.
- Tusiime, G., Adipala, E., Opiyo, F., and Bhagsari, A. S. 1997. Weeds as latent hosts of *Ralstonia solanacearum* in Highland Uganda: Implications to development of an integrated control package for bacterial wilt. Pages 413-419. In: *Bacterial Wilt: Molecular and Ecological Aspects* (Eds. Prior, P., Allen, C., and Elphinstone, J.) INRA Editions, Paris and Springer Germany.

Fig. 1. Typical *Ralstonia solanacearum* colonies on the SMSA semi-selective medium, after five days incubation at 30 °C.

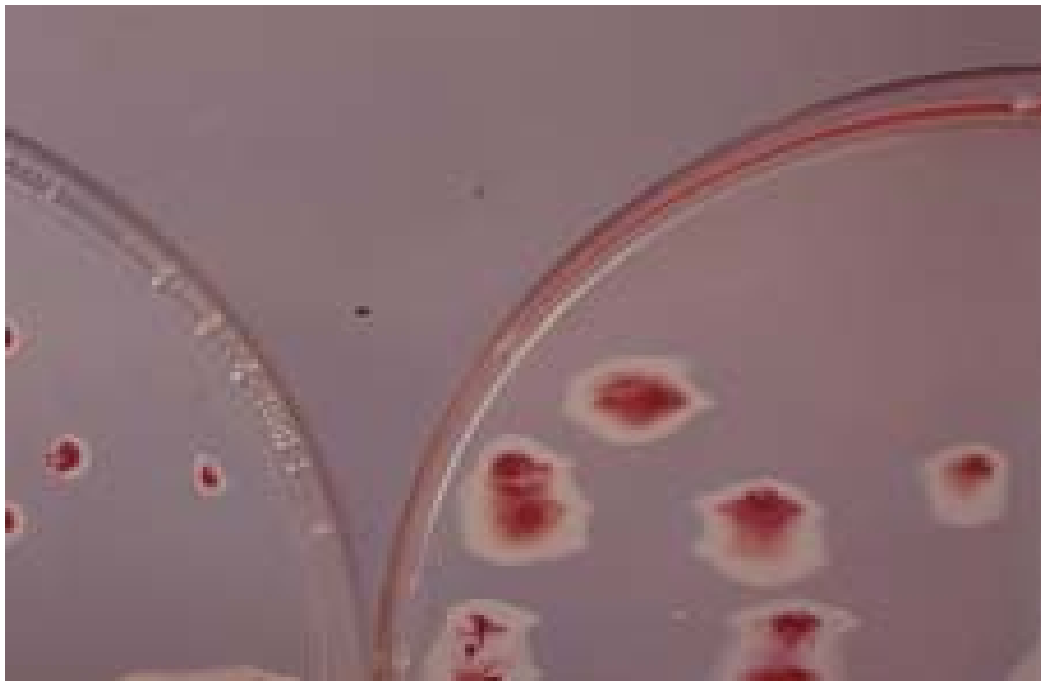


Fig 1.

Fig. 2. The sodium alginate beads containing the pathogen *Ralstonia solanacearum*.



Fig 2.

Fig. 3. Marigolds (*Tagetes patula*) as a possible bio-control agent of *Ralstonia solanacearum*.

Fig. 4. The performance of the tolerant tobacco cultivar in plot A indicates the reduction in the pathogen population in the soil induced by the marigolds/wheat combination, in comparison to the performance of same tobacco cultivar planted in plot B following the tobacco/cabbage treatment.



Fig. 4

Table 1. Schematic representation of the crop rotation trial in a field infested with *Ralstonia solanacearum*.

Treatment 1	Treatment 2	Treatment 3
First summer 1998/1999		
Susceptible tobacco	<ul style="list-style-type: none"> ▪ Early summer - Susceptible tobacco ▪ Late summer - replace tobacco with marigolds, remain on plot during autumn 	Marigolds
First winter 1999		
Cabbage	Plough marigolds in – leave plots fallow throughout winter	Wheat
Second summer 1999/2000		
Susceptible tobacco	<ul style="list-style-type: none"> ▪ Early summer – Susceptible tobacco ▪ Late summer - replace tobacco with marigolds, remain on plot during autumn 	Marigolds
Second winter 2000		
Cabbage	Plough marigolds in - leave plots fallow throughout winter	Wheat
Third summer 2000/2001		
Susceptible tobacco	<ul style="list-style-type: none"> ▪ Early summer – Susceptible tobacco ▪ Late summer - replace tobacco with marigolds, remain on plot during autumn 	Marigolds
Third winter 2001		
Cabbage	Plough marigolds in, leave plots fallow throughout winter	Wheat
Fourth summer 2001/2002		
Tolerant tobacco	Tolerant tobacco cv.	Tolerant tobacco cv.

Table 3. Means of measurements taken of tobacco plants planted on all plots after a three-year crop rotation system with different crop combinations.

Treatment ^a	Means of Measurements			
	Plant survival (%) ^b	Plants with typical symptoms (%) ^b	Plant height (cm) ^b	Green leaf mass / Plant (g) ^b
1 (Tobacco/Cabbage)	92.37 a	15.8 a	57.0 a	353.2 a
2 (Tobacco/Marigolds)	93.00 a	17.9 a	57.7 a	344.2 a
3 (Marigolds/Wheat)	99.00 b	2.3 b	107.3 b	924.2 b
LSD _{Fisher} (P=0.05)	4.136	7.09	10.1	196.6
SEM	1.2	2.1	2.92	56.8
Coefficient of variance (%)	2.5	34.3	7.9	21.0

- ^a
1. Susceptible tobacco cultivar during summer followed by cabbage during winter.
 2. Susceptible tobacco cultivar in early summer – replaced by marigolds for the remainder of summer and throughout fall.
 3. Marigolds during summer followed by wheat during winter

^b Means followed by the same letter do not differ significantly (P > 0.05)

Table 2. Percentage tobacco plants with bacterial wilt symptoms in different rotation systems with various winter crops and marigolds in a greenhouse trial.

Winter crops	First tobacco season				Bio Control Crop	Second tobacco season			
	% Tobacco plants with symptoms					% Tobacco plants with symptoms			
	Weeks after planting					Weeks after planting			
	Four	Five	Seven	Eight		Four	Five	Seven	Eight
Sweet potatoes (<i>Ipomoea batatas</i> L.)	0	33	52	66	Marigolds	17	22	28	39
Onions (<i>Allium cepa</i> L.)	38	62	81	90	Marigolds	6	11	22	39
Coriander (<i>Coriandrum sativum</i> L.)	43	62	81	85	Marigolds	39	67	94	100
Garlic (<i>Allium sativum</i> L.)	47	57	90	95	Marigolds	39	56	78	94
Cabbage (<i>Brassica oleracea</i> L. var <i>capitata</i> Alef).	66	71	100	100	Marigolds	28	50	94	100

SUMMARY

The phase out of well-known chemical control products, especially soil fumigants, is a reality and biological control strategies need to be explored. Biological control rarely eliminates a pathogen from the soil, usually it reduces its numbers or ability to induce disease. However combining complementary biological control tactics may become the only tool in integrated control management for soilborne diseases. The biggest challenge will be to determine the most feasible sequence of techniques for optimal control of each pathogen, farming system and geographic area. Other factors that may have an influence on the efficacy of any bio-control strategy are host related (genetic resistance), environment related (longevity, soil type & climate) and pathogen related (virulence, inoculum density, survival).

There is considerable interest in biofumigation as an alternative to synthetic soil fumigants in horticulture and agriculture. The term biofumigation usually refers to the biocidal effects of isothiocyanates (ITC's) released from Brassicaceous plants. It can and should however be extended to other volatile bio-substances with the same qualities like thiophene, a heterocyclic, sulphurous compound released by *Tagetes* spp.

The rhizosphere presents a complex integrated ecosystem. Biotic and abiotic factors present, can determine the dynamics and structure of the microbial population in a specific rhizosphere. Screening different crops for rhizosphere suppression of soilborne pathogens can be a point of departure to select crops for a specific crop rotation system. A series of summer- and winter crops were evaluated for rhizosphere suppression of *Ralstonia solanacearum*

(Race 1, Biovar III), causal agent of bacterial wilt in tobacco. *Ocimum basilicum* (sweet basil), *Tagetes erecta* (giant marigold), *T. patula* (dwarf marigolds), *Coriandrum sativum* L. (*coriander*) and *Triticum aestivum* L. (wheat cv. Inia) resulted in a significant control of the pathogen. However all *Tagetes* spp. had the lowest pathogen population count of all crops evaluated.

A greenhouse trial conducted in 0,5 m deep soil indicated that the *T. patula* in combination with non-host winter crops, could reduce the pathogen population of the soil to such an extent that the disease incidence on the follow-up tobacco crop was 40% lower than that of the initial tobacco crop. A four-year rotation trial on a heavily infested field showed that *T. patula* in combination with wheat was responsible for a significantly higher tobacco yield (green leaf mass). In both greenhouse and field trials, cabbage (*Brassica oleracea* L. var *capitata*.) proved to be a latent host, suggesting that it should be avoided as a winter cash crop in tobacco fields infested with *R. solanacearum*.

OPSOMMING

Die uifasering van erkende chemiese beheermiddels, veral grond berokings middels, het 'n leemte veroorsaak en het dit nodig geword om biologiese beheer strategieë te ondersoek. Biologiese beheer het selde die totale eliminasië van die patogeen gevolg, maar lei gewoonlik slegs tot 'n vermindering van die patogeenpopulasië of die vermoë daarvan om infeksie te induseer. 'n Kombinasie van ondersteunende biologiese beheermaatreëls mag dalk die enigste instrument wees wat ons sal hê vir geïntegreerde beheer van grondgedraagde-siektes. Die grootste uitdaging lê daarin om 'n uitvoerbare reeks van tegnieke vir optimale beheer van 'n patogeen vir verskillend boerdery sisteme en geografiese areas te bepaal. Ander faktore wat 'n invloed op die uitwerking van biologiese beheer mag hê, is gasheer verwant (genetiese weerstand), omgewings verwant (klimaat & grond tipes) en patogeen verwant (lewensduur, virulensie, innokulum digtheid & oorlewing)

Daar is toenemende belangstelling in bio-beroking as 'n alternatief vir kunsmatige berokingsmiddels in tuin- en landboukunde. The term bio-beroking verwys gewoonlik na biosidiese effekte van isotiosianate wat deur Brassica plants vrygestel word. Dit kan, en behoort egter uitgebrei te word na ander vlugtige, bio-substrate met soortgelyke eienskappe soos bv. tiofeen, 'n heterosikliese, swaelagtige verbinding wat deur *Tagetes* spp. Vrygestel word.

Die risosfeer verteenwoordig 'n komplekse, geïntegreerde ekosisteem. Die teenwoordigheid van biotiese and abiotiese faktore in die risosfeer, kan die kragteleer en strukture van die mikrobiale populasies daarin bepaal. Siftingstoetse van verskillende gewasse t.o.v. risosfeer onderdrukking van

grondgedraagde patogene kan 'n vertrekpunt wees vir die seleksie van gewaskombinasies in wisselboustelsels. 'n Reeks van somer- en wintergewasse is ge-evalueer vir risosfeer onderdrukking van *Ralstonia solanacearum* (Ras 1, Biovar III), die oorsaaklike patogeen van bakteriese verwelksiekte van tabak. *Ocimum basilicum* (soet basielkruid), *Tagetes erecta* (groot afrikaner), *T. patula* (dwerg afrikaner), *Coriandrum sativum* L. (koljander) and *Triticum aestivum* L. (koring, cv. Inia) veroorsaak 'n betekenisvolle beheer van die patogeen. *Tagetes* spp. het egter die laagste patogeen populasie telling gelewer.

'n Glashuisproef in 0,5 m diep grond, het aangedui dat *T. patula* in kombinasie met nie-gasheer wintergewasse die patogeen populasie tot so 'n mate kan verminder dat die siekte voorkoms in die daaropvolgende tabak, 40% laer was. 'n Vier jaar gewasrotasie-proef op 'n hoogs geïnfesteerde land, het getoon dat *T. patula* in kombinasie met koring (cv Inia), verantwoordelik was vir 'n betekenisvolle verhoging in tabak opbrengs (groenmassa). Kool, (*Brassica oleracea* L. var *capitata*.) is in beide die glashuis- en landproef as 'n latente gasheer van *R. solanacearum* aangetoon. Daar word dus aanbeveel dat kool nie as a winter kontant gewas op tabaklande wat met *R. solanacearum* besmet is, geplant moet word nie.