Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Recent Res. Devel. Applied Microbiol. Biotechnol., 2(2005): ISBN: 81-7736-293-3

Biologically based technologies for the suppression of soilborne pathogens of vegetables

Daniel P. Roberts[1], Aref A. Abdul-Baki[1], Inga A. Zasada[2] Susan L. F. Meyer[2] and Waldemar Klassen[3]
[1]Sustainable Agricultural Systems Laboratory and [2]Nematology Laboratory Henry A. Wallace, Beltsville Agricultural Research Center, United States Department of Agriculture – Agricultural Research Service, Beltsville Maryland, U.S.A.; [3]Tropical Research and Education Center University of Florida, Homestead, Florida, U.S.A.

Abstract

There is renewed interest in developing biologically based technologies for the control of soilborne plant-pathogenic fungi and nematodes. Biologically based technologies are thought to be more environmentally friendly than chemical pesticides used in conventional agricultural production systems. Unfortunately, biologically based technologies can be less effective than chemically based methods. Here we present a

Correspondence/Reprint request: Dr. Daniel P. Roberts, Sustainable Agricultural Systems Laboratory Bldg. 001, Rm. 140, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, U.S.A
Email: robertsd@ba.ars.usda.gov

brief overview of our research with microbiologically based biological control and cover crops for the suppression of soilborne plant-pathogenic fungi and nematodes. Emphasis is placed on our development of these technologies as replacements for methyl bromide in vegetable production systems. We also discuss approaches for enhancing the performance of these biologically based technologies through their use in combination.

Introduction

Biologically based technologies have been used in agriculture for centuries [1], with crop rotations and organic amendments to the soil documented in ancient Greece, Rome, and the Americas [2-5]. A paradigm shift in agriculture occurred after World War II, when increased use of crop monoculture and chemicals replaced the more sustainable, biologically based technologies [6, 7]. Two major technological events brought about this change: the introduction of new, large farm machinery, which increased farming efficiency and opened the door to large-scale farming, and the chemical revolution, which introduced agricultural chemical products including commercial fertilizers, plastic mulches, and pesticides. This new farming system, now considered conventional, was based on these mechanical and chemical advances, and soon dominated agriculture in the United States and much of the rest of the world. Conventional systems focus on short-term profitability [8] and depend on high inputs of nonrenewable resources such as commercial fertilizers, pesticides, plastic mulches, and fossil fuel for tillage. These systems lack efficiency with respect to the use of these chemical inputs [9]. As a result, rates of commercial fertilizers beyond those required by the crop continue to be applied [10] and appreciable quantities of pesticides end up in surface and groundwater [11]. In addition, tillage associated with conventional agriculture resulted in soil erosion in over 35% of cultivable land [12], and runoff water carried sediments and nutrients, contaminating offsite water resources.

Need for alternative methods for disease suppression

By the end of the 20th century, the adverse impact of conventional agricultural production systems on the environment concerned growers, consumers, and environmentalists. There is mounting pressure on growers to revert to agricultural practices that are environmentally friendly [13, 14]. Fungicides and nematicides are being re-evaluated with regard to human toxicity and environmental safety. A number of fumigants and other pesticides have been deregistered or are being phased out [9, 15]. This situation has created an urgent need to provide growers with economical alternatives for these chemicals [4]. A striking example is the need to provide growers with

alternatives to the soil fumigant methyl bromide for vegetable production in the United States [16].

For the past four decades, strategies to control soilborne fungal pathogens and nematodes on certain vegetable crops involved preplant soil injection with methyl bromide. Methyl bromide was extensively used because it was economical and effective against a broad spectrum of soil pathogens and weeds. Agriculture in the United States used 32% to 40% of the world production of methyl bromide, which in the early 1990's reached approximately 100,000 tons annually. Based on 1997 data, 83% of the use of methyl bromide in the United States was for preplant use, with California and Florida accounting for 42% and 36%, respectively of the national preplant total [16]. Major vegetable crops which required soil fumigation included tomatoes, peppers, and cucurbits.

The Montreal Protocol was initiated in 1994 to protect the Earth from the detrimental effects of ozone depletion by controlling global production and trade of substances with an ozone depleting potential of 0.2 or higher [17]. Under this treaty, methyl bromide use in vegetable production is to be phased out in the United States. This treaty further stipulated that developing countries will have the advantage of using methyl bromide for an additional ten years after developed countries, such as the United States, have had methyl bromide use phased out.

It was estimated that the ban on methyl bromide use in Florida could result in the loss of $127 million for the winter pepper crop, and a 46% loss in the winter tomato crop [18, 19]. This problem is compounded by competition with Mexico and other countries. From 1990 to 1998, the share of the vegetable market in the United States met by U.S. growers fell from 80% to 70% and that of Florida growers from 35% to 25%. For tomato the loss of market share was greater. In 1992, U.S. and Florida growers supplied 90% and 40% of the U.S. market, respectively; current figures are 67% and 25%. Tomato imports from Mexico increased 200% between 1990 and 1998. The phasing-out of methyl bromide in the United States contributed to this loss of market share as Mexican growers still had access to methyl bromide. Clearly, an environmentally compatible and economically feasible replacement for controlling soilborne plant pathogens and nematodes is needed [20].

A number of chemical alternatives to methyl bromide have been considered for preplant soil fumigation [16]. However, none of these are considered equivalent substitutes. Methyl iodide is most similar to methyl bromide in its biological effects, but is expensive due to inadequate sources for iodide [21]. Biological alternatives to methyl bromide are also being considered [16].

A large portion of our research effort deals with the development of biologically based technologies for use as methyl bromide replacements. Our

approach integrates the use of microbiologically based biological control with cover crops for plant disease management in vegetable production systems. Our research is also directed at furthering our understanding of how these two biologically based technologies work. We discuss here our current efforts to develop biological controls and cover crops for the suppression of soilborne fungal pathogens and nematodes with emphasis on their use as replacements for methyl bromide. This manuscript is not intended as an exhaustive review of the literature.

Biological control for suppression of soilborne pathogens and nematodes

Many microbes suppress populations of soilborne plant pathogens and plant-parasitic nematodes, and consequently reduce adverse effects of these organisms on plants [22, 23]. However, only a small portion of these beneficial microbes are used in commercial agriculture for biological control [24, 25]. There are a number of difficulties associated with the application of these microbes for biological control including inconsistent performance, activity against too few plant pathogens, and failure to act as quickly or as effectively as chemical pesticides [24, 25].

Strategies for biological control of soilborne plant pathogens and plant-parasitic nematodes typically rely on individual microbial biological control isolates for disease suppression [24]. Biological control isolates applied individually will generally be ineffective when several pathogens of the crop are present or under widely varying environmental conditions [25-31]. An approach currently being investigated to overcome these problems is to apply combinations of biological control isolates in a single formulation. Such formulations may be active against multiple pathogens and may have improved activity under diverse soil and rhizosphere conditions [28, 32-34], thereby enhancing disease suppression. Finally, combinations of biological control isolates may allow custom tailored microbial formulations to be targeted against different pathogens of the crop.

The long-term goal of our biological control research is to develop combinations of microbes effective under diverse environmental conditions for management of the plant-parasitic nematode *Meloidogyne incognita* and important soilborne fungal pathogens of cucumber, pepper, and tomato. We screened bacterial and fungal isolates, along with a collection of well-studied *Trichoderma virens* isolates, for suppression of vegetable diseases caused by soilborne plant pathogens [35, 36]. The *T. virens* isolates were originally characterized by G.C. Papavizas, J.A. Lewis, and R.D. Lumsden at the USDA-ARS Beltsville Agricultural Research Center. These isolates suppress soilborne diseases on a number of vegetable crops caused by *M. incognita* and

important plant-pathogenic fungi, including *Rhizoctonia solani* and *Pythium ultimumi* [35-39]. Our strategy is to identify additional antagonists that can be combined with these *T. virens* isolates to improve biological control performance. If newfound isolates have superior disease suppression, they can be developed for application as biological control agents as well.

For example, over fifty bacterial isolates from 21 genera, and *T. virens* Gl-3 and Gl-21, were screened for suppression of damping-off of cucumber caused by *P. ultimum* and *R. solani* in growth chamber and greenhouse assays [36]. Isolates that showed promise for suppression of one or both of these diseases were screened for suppression of *M. incognita* in greenhouse and *in vitro* bioassays. *T. virens* Gl-3 and G1-21 were the most effective for suppression of damping-off caused by *R. solani* and were also effective against *P. ultimum*. Isolates of *Serratia marcescens* were the most effective bacteria against damping-off caused by *P. ultimum*. In addition, *Burkholderia ambifaria* Bc-F was effective against *R. solani* and *P. ultimum*, whereas *Burkholderia cepacia* Bc-1 was effective against *R. solani*. Culture filtrates from most of these isolates inhibited *in vitro* egg hatch of the nematode *M. incognita* [36, 40, 41]. However, none of these isolates consistently suppressed populations of *M. incognita* on cucumber roots [36] despite certain isolates being effective against this nematode on pepper [35].

Certain combinations of these microbial isolates suppressed damping-off of cucumber caused by *R. solani* and *P. ultimum* more consistently than when these isolates were applied individually [36]. For example, the combination of *B. ambifaria* Bc-F with *T. virens* Gl-21 always provided significant biological control of damping-off caused by both pathogens (Table 1). *T. virens* Gl-21 applied alone provided significant suppression of damping-off caused by *R. solani* in two of four experiments and significant suppression of *P. ultimum* in one of three experiments. Likewise, *B. ambifaria* Bc-F applied alone provided significant suppression of *R. solani*, one of two experiments and *P. ultimum* in one of three experiments. Some isolate combinations also showed promise for increasing the level of suppression of damping-off caused by *R. solani* [36]. For example, combining *B. cepacia* Bc-1, applied as a seed treatment, with *T. virens* Gl-21, applied as a granular formulation, increased the level of disease suppression in two of two experiments relative to the individual application of these isolates. Further testing of these combinations against a genetically diverse collection of *P. ultimum* and *R. solani* isolates under a wide range of environmental conditions is required before the full potential of these strain combinations for improved disease suppression is established. Numerous other studies have also reported increased performance by combinations of biological control isolates [reviewed in 25].

Table 1. Suppression of damping-off of cucumber caused by *P. ultimum* or *R. solani* with biological control isolates applied individually or in combination[a].

Treatment	*P. ultimum*[b]	*R. solani*[b]
Trichoderma virens Gl-3	2/3	ND[c]
T. virens Gl-21	1/3	2/4
Burkholderia ambifaria Bc-F	1/3	1/2
B. cepacia Bc-1	0/3	1/2
B. cepacia Bc-2	1/3	ND
Gl-3 + Bc-F	2/3	ND
Gl-3 + Bc-1	2/2	ND
Gl-3 + Bc-2	3/3	ND
Gl-21 + Bc-F	3/3	2/2
Gl-21 + Bc-1	1/3	2/2
Gl-21 + Bc-2	1/3	ND

[a]Experimental details can be found in Roberts et al. [36].
[b]Number of experiments where this treatment provided significant disease suppression relative to the controls/number of experiments performed.
[c]ND, not determined.

Biological control mechanisms and potential for incompatibility

There are examples from our own work, and the work of others, where combinations of microbial isolates resulted in decreased disease suppression or had other problems with compatibility among strains in the formulation [25, 35, 36]. Bell pepper seedlings were treated with *Burkholderia cepacia* Bc-2, *B. ambifaria* Bc-F, and *T. virens* Gl-3 individually, or in combinations, to compare effects of these isolates on populations of *M. incognita*. Populations of *M. incognita* were significantly lower than the untreated control with treatments containing isolates Bc-2, Bc-F, or Gl-3. Treatments combining these isolates (Bc-F + Gl-3, Bc-2 + Gl-3, Bc-F + Bc-2, and Bc-F + Bc-2 + Gl-3) did not reduce populations of *M. incognita* on pepper roots relative to the untreated controls [35]. In studies with damping-off of cucumber discussed above, certain combinations of isolates were incompatible when co-incubated for 10 to 12 days in cucumber rhizosphere [36]. Populations of *T. virens* Gl-3 and Gl-21 (approximately 6.0 $\log_{10}$ CFU) were added to cucumber seeds along with approximately 7.5 $\log_{10}$ CFU *B. cepacia* BC-1 or *S. marcescens* isolates N1-14 or N2-4. Populations of *T. virens* GL-3 and GL-21 could not be detected after 10 to 12 days in cucumber rhizosphere when coincubated with *B. cepacia* BC-1 or with either *S. marcescens* isolate. In contrast, approximately 6.5 $\log_{10}$ CFU *T. virens* Gl-3 or Gl-21 were detected when these fungal isolates were applied alone.

Clearly not all combinations of biological control isolates result in improved and consistent disease suppression. Several researchers have indicated that isolates combined in biological control formulations must be compatible for increased disease suppression to occur [29, 42-44]. There has been considerable interest in determining how various mechanisms, expressed by the biological control agent, result in disease suppression. However, there has been little work regarding how these mechanisms result in incompatibility among microbes combined in a formulation. Microbial biological control mechanisms leading to disease suppression include antibiosis, mycoparasitism or lytic activity, and competition for resources or niche exclusion [24]. A new phase of our work addresses these underlying mechanisms and the role they play in incompatibility among biological control isolates combined in formulations.

Antibiosis. Antibiotics are a diverse group of low molecular weight molecules that are deleterious to the growth or metabolic activity of other organisms [45]. Considerable potential exists for antagonism between microbes combined in biological control formulations due to antibiosis [25, 28]. Antibiotics have been shown to be produced by biological control agents in the rhizosphere and on plant surfaces, regions potentially cohabitated by biological control agents when applied in combination. The potential for antagonism can be great because certain biological control agents produce several antibiotics, some of which have broad spectrum antimicrobial activity [45]. For example, *Pseudomonas fluorescens* PF-5 produces pyrrolnitrin, the polyketide antibiotics pyoluteorin and 2,4-diacetylphloroglucinol, and hydrogen cyanide [46-49]. Pyrrolnitrin, for one, has been shown to be inhibitory to a number of fungi [50]. Several other compounds produced by biological control isolates such as gliotoxin, carbapenem, and prodigiosin have broad spectrum activity against microorganisms [51-53].

Mycoparasitism and hyphal lysis. Mycoparasitism and hyphal lysis also have the potential to result in antagonism among isolates combined in biological control formulations. Fungal cell walls, including the cell walls of fungal biological control agents, consist primarily of carbohydrate with lesser quantities of protein and other compounds [54]. Carbohydrate polymers of chitin or B-glucans make up microfibrillar components of fungal cell walls, while protein and polymers of various other carbohydrates bind the different structural components of the wall into macromolecular complexes [55]. *Serratia*, *Bacillus*, and *Streptomyces* are genera of biocontrol prokaryotes shown to produce multiple isoforms of chitinases which have been implicated in hyphal lysis [56]. Biological control fungi, such as *Trichoderma* sp., have been shown to produce lytic enzymes including chitinase and glucanase which inhibit fungal spore germination and cause abnormal hyphal growth [57, 58].

Competition. Antagonism between biological control isolates within a particular formulation may also arise due to competition for limiting resources, such as nutrients, in the rhizosphere. Various approaches have demonstrated that the rhizosphere can be limiting in reduced carbon, nitrogen, or iron [59-62]. Competition for these limiting resources may result in one of the biological control isolates in the formulation being limited in activity or in population growth [63]. The importance of nutrient limitation in the rhizosphere is exemplified by studies evaluating the role of siderophores which function to sequester and deliver Fe^{3+} [64-66]. Siderophore-mediated competition for iron has been shown to effectively suppress populations and activity of microbes in the rhizosphere [67, 68].

Potential strategies to enhance compatibility

With our approach to use strain combinations for enhanced disease suppression, it is necessary to understand how various compounds produced by the biological control isolates lead to inhibition of the pathogen, and to determine how these compounds affect compatibility of combined biological control strains. As discussed above, we identified bacterial isolates that effectively suppress damping-off of cucumber caused by *P. ultimum* and/or *R. solani*. The related isolates *B. cepacia* Bc-1, *B. ambifaria* Bc-F, and *B. cepacia* Bc-2 strongly inhibited, slightly inhibited, and had no effect, respectively, on populations of *T. virens* Gl-21 when in cucumber rhizosphere. As discussed earlier, *S. marcescens* isolates N2-4 and N1-14 strongly inhibited *T. virens* Gl-21 when co-incubated with this fungus in cucumber rhizosphere [36]. Biological control performance was enhanced with certain combinations (Bc-F + Gl-21; Bc-1 + Gl-21) but not with other combinations (Bc-2 + Gl-21; various *S. marcescens* isolates + Gl-3 or Gl-21). We are investigating the cause of this incompatibility so that strategies can be devised to overcome the antagonism and potentially enhance biological control performance of these strain combinations.

We are currently identifying antibiotics and other inhibitory metabolites produced by these bacterial isolates. A number of inhibitory compounds have been characterized from cultures of *Burkholderia* and *Serratia* isolates including pyrrolnitrin, altericideins, oocydin A, carbapenem, prodigiosin, and serrawettin [69-75]. We are determining if our isolates contain biosynthetic genes for these metabolites using PCR with primers designed from conserved sequences of genes reported in the literature [76]. Confirmation of production of these compounds comes from TLC or HPLC analysis of culture supernatants or cellular extracts. This approach has been used to confirm production of pyrrolnitrin by *B. ambifaria* Bc-F and *B. cepacia* isolates Bc-1 and Bc-2 (Roberts et al., *unpublished*). Bioautography will be used to look for uncharacterized inhibitory compounds from cultures of our isolates grown on

different substrates extracted with various solvent systems [77]. In the second step, mutants deficient in the production of these inhibitory compounds will be isolated using classical transposon mutagenesis procedures [78]. The role of these compounds in disease suppression will then be assessed using biological control assays where disease suppression by the mutant and wild-type strains will be compared. The role of the metabolite in compatibility will also be determined by co-incubating mutant isolates and *T. virens* in cucumber rhizosphere.

If the inhibitory compound is not important for disease suppression but does result in incompatibility, a derivative strain that does not produce the compound can be used in biological control formulations. If the compound is important for disease suppression, then other strategies to overcome incompatibility can be employed. For example, incompatibility among biological control isolates was surmounted resulting in enhanced disease suppression in work by DeBoer et al. [79]. *Pseudomonas fluorescens* RS111 was strongly inhibited *in vitro* by *Pseudomonas putida* RE8 while strain RE8 was not inhibited by strain RS111. The inhibition was caused by a compound, possibly an antibiotic, which diffused into the agar medium. A spontaneous mutant, strain RS111a, was isolated that was less sensitive than strain RS111 to inhibition by strain RE8. Compatible and incompatible combinations of these strains were tested for control of Fusarium wilt of radish. Application of the compatible strain combination (RS111a + RE8) resulted in enhanced disease suppression when compared with the pathogen control, the strains applied individually, and the incompatible strain combination (RS111 + RE8).

Competition for reduced carbon nutrients among microbes resulting in antagonism was shown by Wilson and Lindow [80, 81]. Strains with high reduced carbon utilization overlap were antagonistic with each other through competition for reduced carbon. Conversely, coexistence of bacterial species on leaf surfaces was mediated through the utilization of different reduced carbon nutrients by coexisting strains [81]. This suggests that biological control isolates with low reduced carbon utilization overlap should be compatible in the rhizosphere.

We have developed a collection of nutritional mutants of *Enterobacter cloacae* 501R3 to examine the role of reduced carbon compounds released from seeds and roots in colonization and disease suppression by this bacterium (Table 2). Certain mutants lost the ability to utilize whole categories of reduced carbon compounds that were detected in cucumber exudates. Strain A-11, with a mutation in *pfkA* [82], lost the ability for wild-type growth on almost all carbohydrate compounds in cucumber exudates, while strain M2 [78] lost the ability for wild-type growth on most amino acids and organic acids in cucumber exudates. There was only a slight reduction in colonization of the cucumber rhizosphere by strains A-11 and M2 relative to the wild-type

strain, 501R3, despite these decreased abilities to grow on compounds in cucumber exudates (Roberts et al., *unpublished*). Only strain M43, which has lost the ability to utilize almost all compounds detected in cucumber seed and root exudates, showed a dramatic decrease in colonization of cucumber rhizosphere relative to strain 501R3 (Roberts et al., *unpublished*). Preliminary experiments with these mutants examining suppression of damping-off of cucumber caused by *P. ultimum* indicate that utilization of these reduced carbon compounds by *E. cloacae* is not important for disease suppression. All mutants resulted in disease suppression similar to strain 501R3 in these preliminary experiments (Roberts et al., *unpublished*).

Table 2. Colonization and disease suppression phenotypes of mutants of *Enterobacter cloacae.*

Mutant	Gene	Nutritional deficiency[a]	Root colonization[b]	Disease suppression[c]
A-11	*pfkA*	Most carbohydrates	Moderately reduced	Wild-type
M2	*sdhA*	Most amino acids, TCA cycle intermediates	Moderately reduced	Wild-type
M43	*aceF*	Most carbohydrates, amino acids, TCA cycle intermediates	Deficient	Wild-type

[a]Growth of mutant strain versus wild-type strain 501R3 on individual reduced carbon compounds detected in cucumber seed and root exudates.
[b]Populations of mutant and wild-type strain 501R3 on roots were compared at various times in experiments conducted with cucumber grown in potting mix and in natural soil.
[c]Mutant strains and wild-type strain 501R3 were compared for suppression of damping-off of cucumber caused by *Pythium ultimum* in growth chamber bioassays conducted in potting mix.

These results suggest that *E. cloacae* is flexible with regard to reduced carbon nutrition during colonization of cucumber seeds and roots and suppression of damping-off. In situations where competition for reduced carbon between strains in biological control formulations results in antagonism, it may be possible to increase coexistence using mutants that utilize mutually exclusive sets of reduced carbon nutrients. These mutants can be derived through genetic engineering (Table 2). It may also be possible to select for spontaneous mutants that have lost the ability to grow on categories of reduced carbon found in exudates [83].

Spatial or temporal separation of species within a community has also been shown to improve coexistence of members of the community [84, 85]. There are a number of potential ways to facilitate separation of biological control isolates combined in a formulation. Root interiors represent additional habitats, spatially distinct from the rhizosphere. One isolate might be selected

for rhizosphere competence, another for ability to grow internally within plant tissues [86]. However, there has been little work done in this area. It may also be possible to separate biological control isolates at the time of application to the plant to improve compatibility of these isolates. As discussed above, we applied bacterial biological control isolates as seed coatings, in combination with a granular formulation of *T. virens* applied to the planting medium, for suppression of *R. solani* on cucumber [36]. Some of these bacterial antagonists combined with *T. virens* resulted in enhanced disease suppression despite being incompatible in cucumber rhizosphere. For example, suppression of *R. solani* was improved when *T. virens* Gl-21 was combined with *B. cepacia* Bc-1 [36]. Finally, certain diseases do not require long-term persistence of the biological control agent in association with the plant. Such is the case for damping-off of cucumber caused by *P. ultimum* [87]. It may be possible to enhance performance of a formulation by combining a non-persistent biological control isolate, targeted against *P. ultimum*, with a biological control isolate that is effective in colonization of the cucumber rhizosphere and targeted against a second pathogen.

Cover crops for suppression of soilborne pathogens and nematodes

Biological control focuses on the management of soilborne pathogens and does not deal with other serious problems associated with vegetable production, such as soil erosion, loss of soil fertility, contamination of surface and groundwater with fertilizers, and the depletion of natural resources. The use of cover crops has reduced soil erosion, reduced water and nutrient run-off, improved physical soil characteristics, reduced plant disease, and increased soil organic matter [reviewed in 9, 88-90]. The incorporation of appropriate legume cover crops to the production rotation can also result in substantial quantities of nitrogen fixed and recycled by the cover crop. The interactions among the components of a cover cropping system (reduced tillage, legume cover crops, and crop rotations) result in a fertile soil with minimum chemical inputs and low management costs. The following is a brief description of a cover crop-based production system developed for south Florida where *M. incognita* is the most serious pest limiting production of tomatoes and other vegetables. For decades, *M. incognita* and other soil pathogens were controlled by soil fumigation with methyl bromide and chloropicrin or the use of resistant vegetable cultivars.

There are a number of nematode-resistant vegetable cultivars available. However, they all have their limitations. Commercially available cultivars of tomato include Sanibel, Sunjay, Clemente, Cisco, Shady Lady, and Roger 6153. All of these tomato cultivars have the *Mi* gene that is effective at

temperatures below 31.5° C [91]. At temperatures above 31.5° C, tomato plants lose resistance to *M. incognita*. Because of this concern alternative disease management practices are needed.

A large number of grassy and leguminous cover crop cultivars have shown resistance to one or more species of plant-parasitic nematodes [92-98]. Unfortunately, no one cover crop serves as a non-host to all nematode species. Therefore, in soils where many nematode species are found, a cover crop should be selected that targets the species most damaging to the vegetable crop. In the tomato growing area of south Florida, *M. incognita* causes the most damage and, consequently, the non-host leguminous cover crops velvetbean (*Mucuna pruriens* cv. Utilis), cowpea (*Vigna unguiculata* cv. Iron Clay) and sunn hemp (*Crotalaria juncea* cv. Tropic Sun) were used. All three of these legume species performed well in the tropical climate of south Florida. They produced 6 to 12 tons of biomass per hectare with a nitrogen content of about 2.5 % to 3%.

The cover crops-based system was evaluated in large-scale field trials over three years in south Florida. Soils in these trials were infested with low to moderate levels of nematodes and had not been treated with fumigants (Abdul-Baki et al., *unpublished*). Soil was prepared in summer, and cover crops were seeded in raised beds following a procedure described elsewhere [99]. The cover crops were flail mowed and their biomass residue was incorporated into the soil. Raised beds were subsequently reformed and seedlings of the *M. incognita*-resistant tomato cultivars 'Sanibel' and 'Roger 6153' were transplanted. A standard soil fumigation treatment using methyl bromide plus chloropicrin was included each year for comparison. Tomato yields in all three years of these trials averaged higher than average yields in Miami-Dade County. Yields from the cover crops-based treatments were equal to, or greater than, those from the methyl bromide plus chloropicrin treatment in two of three years. A subsequent economic analysis, taking into account production costs, showed a higher net return from the biologically based treatments than from the methyl bromide plus chloropicrin treatment in all years. This was due to a savings of $1544 per hectare in the biologically based treatment over the methyl bromide plus chloropicrin treatment (Abdul-Baki et al., *unpublished*). Though the tests were limited to three years, it is reasonable to conclude that in soils where *M. incognita* populations are moderate to low, soil fumigation can be skipped and the cover crops-based system can serve as an economically viable and environmentally safe substitute to the methyl bromide-based system.

Combining biological control with cover crops

A wide range of cover crops suppressive to soilborne pathogens have been evaluated [100]. As with biological control with microorganisms, consistent

suppression of soilborne plant pathogens is sometimes lacking. This is due, in part, to our incomplete understanding of the mechanisms by which cover crops suppress these pathogens. Research needs to be conducted investigating the pathogen host status of cover crops, the chemical composition of the active components released from cover crops, the lethal concentration values of these active components for specific target pathogens, the impact of this material on soil physical and chemical properties, and the influence and impact of soil biotic factors on disease suppression by cover crops. In addition, developing new cultivars of cover crops with resistance to nematodes should be a major focus of plant breeders.

Combining microbiologically based biological control with cover crops may improve consistency of disease suppression relative to either technology applied individually. Potential advantages resulting from combining these technologies are similar to those mentioned above regarding the combination of biological control isolates. Such combinations are more likely to have a greater variety of mechanisms responsible for suppression of one or more pathogens and also are likely to have these mechanisms expressed over a wide range of environmental conditions. Combining these technologies may also broaden the spectrum of activity and increase the level of disease suppression. Additionally, combinations may allow custom tailoring for suppression of specific pathogens present in a particular field.

Whereas little information exists regarding the combined application of cover crops and biological control agents, evidence does exist that this approach has potential. The prolonged use of cover crops enhanced nematode-trapping fungi compared to weeds [101]. Nematode-antagonistic bacteria and chitinolytic fungi were present at higher densities in the rhizosphere of nematode-suppressive cover crops [102]. Microbial enhancement by cover crops may be a result of increased soil organic matter, providing a favorable environment for microbial activity or removing soil microbiostasis [103, 104]. Based upon the enhancement of indigenous antagonistic microbes by cover crops, the same effect may be achieved when combining biological control agents with cover crops.

Conclusions

Biologically based technologies have potential for use in commercial agriculture for suppression of soilborne plant-pathogenic fungi and plant-parasitic nematodes of vegetable crops. The biologically based system has received acceptance from organic growers (who can not use soil fumigants) and from small-scale growers located close to residential areas. Large-scale growers will remain reluctant to use these biologically based technologies until they are demonstrated to be economical and consistently effective. Ecologically based arguments suggest that combining these technologies will

enhance disease suppression performance. There is some evidence that suggests that combining microbial biological control isolates can improve disease suppression. Ecological principles suggest that combining biological control microbes with cover crops also should improve disease suppression performance. However, as with all biologically based technologies, we need to deepen our understanding of the mechanisms resulting in disease suppression. This is necessary so that strategies can be devised to allow these mechanisms to preferentially target the pathogenic microbial population as opposed to the beneficial biological control microbes.

References

1. Bailey, K.L., and Lazarovits, G. 2003, Soil Till. Res., 72, 169.
2. Barker, K.L, Lafond, G.P., and Domitruk, D. 1998, Can J. Plant Sci., 81, 789.
3. Karlen, D.L., Varvel, G.E., Bullock, D.G., and Cruse, R.M. 1994, Adv. Agron., 53, 1.
4. Lazarovits, G. 2001, Can. J. Plant Pathol., 23, 1.
5. Rodriguez-Kabana, R., Morgan-Iones, G., and Chet, I. 1987, Plant Soil, 100, 237.
6. Katan, J., 1996, Management of Soilborne Diseases, R.S. Utkhede and V.K. Gupta (Eds.), Kalyani Publishers, New Delhi, p. 100.
7. Hague, N.G.M., and Gowen, S.R. 1987, Principles and Practices of Nematode Control in Crops, R.H. Brown and B.R. Kerry (Eds.), Academic Press, Ireland, 447.
8. Gillespie, G.W., Jr., Lyson, T.A., and Power, A. 1995, Sustainable Agr., 7, 5.
9. Abawi, G.S., and Widmer, T.L. 2000, Applied Soil Ecol., 15, 37.
10. Association of American Plant Food Officials. 2000, Commercial Fertilizer 2000. Division of Regulatory Services. Univ. Kentucky, Lexington, KY., p. 4.
11. Aspelin, A.L. 1997, Pesticides Industry Sale and Usage: 1994 and 1995 Market Estimates. U.S. Environmental Protection Agency, Washington, D.C.
12. National Research Council. 1989. Alternative Agriculture. Natl. Academy Press, Washington, D.C.
13. Akhtar, M., and Mahmood, I. 1995, Int. Pest Contrl., 37, 86.
14. Duncan, L.W. 1991, Annu. Rev. Phytopathol., 29, 467.
15. Gamliel, A., Austerweil, M., and Kritzman, G. 2000, Crop Prot., 19, 847.
16. Martin, F.N. 2003, Annu. Rev. Phytopathol., 41, 325.
17. U.S. EPA Methyl Bromide Phase Out Website 1/28/02.
18. Gilreath, J. P., Jones, J.P., and Overman, A.J. 1994, Proc. Florida State Hort. Soc., 107, 156.
19. Sanchez, P. 1997, Ag. Research, 45, 12.
20. Kim, K.D., Nemec, S., and Musson, G. 1997, Appl. Soil Ecol., 5, 169.
21. Gilreath, J.P., and Santos, B.M. 2004, Crop Prot. 28, 1193.
22. Kerry, B.R. 2000, Annu. Rev. Phytopathol., 38, 423.
23. Siddiqui, I.A., and Shaukat, S.S. 2003, Nematol. Medit., 31, 111.
24. Larkin, R.P., Roberts, D.P., and Gracia-Garza, J. 1998, Fungicidal Activity. Chemical and Biological Approaches to Plant Protection, D. Hutson and J. Miyamoto (Eds.), p. 149.

25. Meyer, S.L.F., and Roberts, D.P. 2002, J. Nematol., 34, 1.
26. Boeger, J.M., Chen, R.S., and McDonald, B. 1993, Phytopathology, 83, 1148.
27. Kerry, R.B., and Bourne, J.M. 1996, Pestic. Sci., 47, 69.
28. Pierson, E.A., and Weller, D.M. 1994, Phytopathology, 84, 940.
29. Raupach, G.S., and Kloepper, J.W. 1998, Phytopathology, 88, 1158.
30. Sikora, R.A., and Hoffman-Hergarten, S. 1993, Pest Management: Biologically Based Technologies, R.D. Lumsden and J.L. Vaughn (Eds.), American Chemical Society, Washington, D.C., p. 166.
31. Stirling, G.R. 1991, Biological Control of Plant-Parasitic Nematodes. CAB International, Wallingford, U.K.
32. Lemanceau, P. and Alabouvette, C. 1991. Crop Prot., 10, 279.
33. Lemanceau, P., Bakker, P.A.H.M., de Kogel, W.J., Alabouvette, C., and Schippers, B. 1993, Appl. Environ. Microbiol., 58, 2978.
34. Crump, D.H. 1998, Aspects Appl. Biol., 53, 383.
35. Meyer, S.L.F., Roberts, D.P., Chitwood, D.J., Carta, L.K., Lumsden, R.D., Mao, W. 2001, Nematropica, 31, 75.
36. Roberts, D.P., Lohrke, S.M., Meyer, S.L.F., Buyer, J.S., Bowers, J.H., Baker, C.J., Li, W., de Souza, J.T., Lewis, J.A., and Chung, S. 2005, Crop Prot., 24, 141.
37. Lewis, J.A., and Lumsden, R.D. 2001, Crop Prot., 20, 49.
38. Mao, W., Lewis, J.A., Lumsden, R.D., and Hebbar, K.P. 1998, Crop Prot., 17, 535.
39. Papavizas, G.C. 1985, Annu. Rev. Phytopathol., 23, 23.
40. Meyer, S.L.F., Massoud, S.I., Chitwood, D.J., and Roberts, D.P. 2000, Nematology, 2, 871.
41. Li, W., Roberts, D.P., Dery, P.D., Meyer, S.L.F., Lohrke, S.M., Lumsden, R.D., and Hebbar, K.P. 2002, Crop Prot., 21, 129.
42. Baker, R. 1990, Biological Control of Soil-borne Plant Pathogens, D. Hornby (Ed.), CAB International, Wallingford, UK, p. 375.
43. Janisiewicz, W., and Bors, B. 1995, Appl. Environ. Microbiol., 61, 3261.
44. Janisiewicz, W. 1996, Phytopathology, 86, 473.
45. Duffy, B., Schouten, A., and Raaijmakers, J. 2003, Annu. Rev. Phytopathol., 41, 501.
46. Howell, C.R., and Stipanovic, R.D. 1980, Phytopathology, 70,712.
47. Howell, C.R., and Stipanovic, R.D. 1979, Phytopathology, 69, 480.
48. Kraus, J., and Loper, J.E. 1992, Phytopathology, 82, 264.
49. Nowak-Thompson, B., Gould, S.J., Kraus, J., and Loper, J.E. 1994, Can. J. Microbiol., 40, 1064.
50. Lignon, J.M., Hill, D.S., Hammer, P.E., Torkewitz, N.R., Hofmann, D. 2000, Pest Manag. Sci., 56, 688.
51. Bennet, J.W., and Bentley, R. 2000, Adv. Appl. Microbiol., 47, 1.
52. Jones, R.W., and Hancock, J.G. 1988, J. Gen. Microbiol., 134, 2067.
53. McGowan, S.J., Holden, T.G., Bycroft, B.W., and Salmond, G.P.C. 1999, Ant. Van Leeuw., 75, 135.
54. Peberdy, J.F. 1990, Biochemistry of Cell Walls and Membranes in Fungi, P.J. Kuhn (Ed.), Springer-Verlag, Heidelberg, Germany, p. 5.
55. Bartnicki-Garcia, S. 1968, Annu. Rev. Microbiol., 22, 87.

56. Roberts, D.P., and Kobayashi, D.Y. 2001, Encyclopedia of Plant Pathology, O.C. Maloy and T.D. Murray (Eds.), John Wiley and Sons, New York, p. 558.
57. Lorito, M., Di Pietro, A., Hayes, C.K., Woo, S.L., and Harman, G.E. 1993, Phytopathology, 83, 302.
58. Lorito, M., Hayes, C.K., Di Pietro, A., Woo, S.L., and Harmen, G.E. 1994, Phytopathology, 84, 398.
59. Chen, W., Hoitink, H.A., Schmitthenner, F., and Tuovinen, O. 1988, Phytopathology, 78, 314.
60. Kloepper, J.W., Leong, J., Teintze, M., and Schroth, M.N. 1980, Curr. Microbiol., 4, 317.
61. Loper, J.E. 1988, Phytopathology, 78, 166.
62. Weller, D.M., Howie, W.J., and Cook, R.J. 1988, Phytopathology, 78, 1094.
63. Paulitz, T.P., and Baker, R. 1987, Phytopathology, 77, 335.
64. Loper, J.E., and Henkels, M.D. 1999, Appl. Environ. Microbiol., 65, 5357.
65. Mirleau, P., Philippot, L., Corberand, T., and Lemanceau, P. 2001, Appl. Environ. Microbiol., 67, 2627.
66. Raaijmakers, J.M., van der Sluis, I., Koster, M., Bakker, P.A.H.M., Weisbeck, P.J., and Schippers, B. 1995, Can. J. Microbiol., 41, 126.
67. Leong, J. 1986, Annu. Rev. Phytopathol., 24, 187.
68. Loper, J.E., and Buyer, J.S. 1991, Molec. Plant-Microbe. Interact., 4, 5.
69. Asano, S., Ogiwara, K., Nakagawa, Y., Suzuki, K., Hori, H., and Watanabe, T. 1999, J. Pest. Sci., 24, 381.
70. Kamensky, M., Ovadis, M., Chet, I., and Chernin, L. 2003, Soil Biol. Biochem., 35, 323.
71. Kirinuki, T., Ichiba, T., and Katayama, K. 1984, J. Pestic. Sci., 9, 601.
72. Lindum, P.W., Anthoni, U., Christophersen, C., Eberl, L., Molin, S., and Givskov, M. 1998, J. Bacteriol., 180, 6384.
73. McGowan, S.J., Holden, T.G., Bycroft, B.W., and Salmond, G.P.C. 1999, Ant. Van Leeuw., 75, 135.
74. Roitman, J.N., Mahoney, N.W., Janiscwicz, W.J., and Benson, M. 1990, J. Agric. Food Chem., 38, 538.
75. Strobel, G., Li, J.Y., Sugawara, F., Koshino, H., Harper, J., and Hess, W.M. 1999, Microbiol., 145, 3557.
76. de Souza, J.T., Mazzola, M., and Raaijmakers, J.M. 2003, Environ. Microbiol., 5, 1328.
77. Burkhead, K.D., Schisler, D.A., and Slininger, P.J. 1995, Soil Biol., Biochem., 27, 1611.
78. Roberts, D.P., Marty, A.M., Dery, P.D., Yucel, I., and Hartung, J.S. 1996, Soil Biol. Biochem., 28, 1015.
79. de Boer, M., van der Sluis, I., Van Loon, L.C., and Bakker, P.A.H.M. 1997, Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects, A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, and S. Akino (Eds.), OECD Workshop, Japan, p.380.
80. Wilson, M., and Lindow, S.E. 1994, Appl. Environ. Microbiol., 60, 3128.
81. Wilson, M., and Lindow, S.E. 1994, Appl. Environ. Microbiol., 60, 4468.
82. Roberts, D.P., Dery, P.D., Yucel, I., Buyer, J., Holtman, M.A., and Kobayashi, D.Y. 1999, Appl. Environ. Micribiol., 65, 2513.

83. Roberts, D.P., Dery, P.D., and Hartung, J.S. 1996, Soil Biol. Biochem., 28, 1109.
84. Evans, D.R., Hill, J., Williams, T.A., and Rhodes, I. 1989, Theor. Appl. Genet., 77, 65.
85. Niemela, J. 1993, Oikos, 66, 325.
86. Chao, W.L., Nelson, E.B., Harman, G.E., and Hoch, H.C. 1986, Phytopathology, 76, 60.
87. Roberts, D.P., Dery, P.D., Hebbar, K.P., Mao, W.L., and Lumsden, R.D. 1997, J. Phytopathol., 145, 387.
88. Abdul-Baki, A.A., Teasdale, J.R., Korcak, R.F., Chitwood, D.J., and Huettel, R.N. 1996, HortScience, 31, 65.
89. Abdul-Baki, A.A., Teasdale, J.R., Goth, R.W., and Haynes, K.G. 2002, HortScience 37, 878.
90. Rice, P.J., McConnell, L.L., Heighton, L.P., Sadeghi, A.M., Isensec, A.R., Teasdale, J.R., Abdul-Baki, A.A., Harman-Fetcho, J.A., and Hapeman, C.J. 2001, J. Environ. Qual., 30, 1808.
91. Abdul-Baki, A.A., Haroon, S.A. and Chitwood, D.J. 1996, J. Amer. Soc. Hort. Sci., 31, 147.
92. Aguiar, J., Ehlers, J., and Graves, W. 1998, California Vegetable Journal, November/December, 5.
93. Araya, M. and Caswell-Chen, E.P. 1994, J. Nematol., 26, 492.
94. DuFour, R., Earles, R., Kuepper, G., and Greer, L. 1998, Appropriate Technology Transfer for Rural Areas (ATTRA), January, 1.
95. McSorley, R. 2000, Annual Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions. 6-9 Nov., 2000, Orlando, Florida.
96. McSorley, R., Campbell, K.L., Graham, W.D., and Del-Bottcher, A.B. 1994, Proc. Second Conf. Amer. Soc. Agr. Engineers, W.D. Graham (Ed.), p. 517.
97. Rodriguez-Kabana, R., Pinochet, J., Robertson, D.G. and Wells, L.W. 1992, J. Nematol. (Suppl.), 24, 662.
98. Vargas-Ayala, R., and Rodriguez-Kabana, R. 2001, Nematropica, 31, 37.
99. Abdul-Baki, A.A., Bryan, H., Klassen, W., Carrera, L., Li, Y.C., and Wang, Q. 2004, Acta Hort., 638, 419.
100. Halbrendt, J.M. 1996, J. Nematol., 28, 8.
101. Wang, K.-H., Sipes, B.S., and Schmitt, D.P. 2003, J. Nematol., 35, 39.
102. Kloepper, J.W., Rodriguez-Kabana, R., McInroy, J.A., and Collins, D.J. 1991, Plant Soil, 136, 95.
103. Ho, W.C., and Ko, W.H. 1986, J. Gen. Microbiol.,132, 2807.
104. Mankau, R., and Minteer, R.J. 1962, Plant Dis. Rprtr., 46, 375.