

PROMOTING PLANT HEALTH: POTENTIAL FOR THE USE OF PLANT-ASSOCIATED MICRO-ORGANISMS IN THE BIOLOGICAL CONTROL OF PATHOGENS AND PESTS IN SUGARCANE

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Abstract

Bacteria and fungi that colonise plant surfaces and internal plant tissues (epiphytes and endophytes respectively) are ubiquitous, residing latently or actively colonising the plant locally as well as systemically. Endophytic microorganisms generally originate from the epiphytic communities of the root or aerial surfaces as well as from within seed material. Besides entering plants through natural openings or wounds, many endophytes appear capable of actively penetrating their hosts using hydrolytic cellulases and pectinases. In these respects endophytes resemble pathogens.

Historically many of these microorganisms have been thought to be weakly virulent plant pathogens. However, some have since been discovered to have beneficial effects on host plants, such as growth promotion and increased resistance against plant pathogens, insect pests and nematodes. Increased resistance can occur through competitive exclusion, direct antibiosis and induced systemic resistance in the plant, or combinations of these. Conversely, some epiphytes and endophytes might be regarded as plant antagonists in that they retard plant growth and increase susceptibility to pathogens, pests and nematodes.

This review assesses the potential for the biological control of important sugarcane pathogens and pests using beneficial epiphytic and endophytic microorganisms based on results achieved with other crop plants.

Keywords: Biocontrol, *Burkholderia*, *Eldana*, *Fusarium*, Mycorrhizae, Trichoderma.

Introduction

Pathogens invade the tissues of the plant and form parasitic relationships. At the extremes, these relationships are either obligately biotrophic, where the host tissue is kept alive and nutrients are extracted from it (e.g. *Ustilago scitaminea* – sugarcane smut) (Schenk, 1998), or necrotrophic, where the host tissue is first killed and the pathogen feeds on the dead material (e.g. *Ceratocystis paradoxa* – pineapple disease of sugarcane) (Girard and Rott, 2000). Because they cause obvious or major symptoms, and nearly always result in a reduction in yield, pathogens such as these may be referred to as major pathogens.

However, not all pathogens cause obvious symptoms. Minor pathogens parasitise tissues such as root tips and root hairs causing localised injury and some loss of function (Salt, 1979). Major pathogens can also act as minor pathogens when conditions are suboptimal for full disease development. For example, *Pythium* species are ubiquitous in soils planted to sugarcane and can cause severe rotting of young roots during cool wet conditions. Under suboptimal conditions for the pathogen, root injury occurs to a lesser extent and presumably this leads to unrecognised yield losses. *Pythium* has been linked to the phenomenon known as yield decline (Hoy and Schneider, 1988a,b).

Another class of minor pathogens consists of non-parasitising exo-pathogens (Schippers, *et al.* 1987; Suslow and Scroth, 1982). Most of these colonise the root surface or rhizosphere, apparently without infecting the roots. This group includes both bacteria (deleterious rhizobacteria) and fungi which antagonise the roots thereby suppressing growth and yield without producing any other obvious symptoms (Wolz, 1978).

Microorganisms that associate with plants are not all unfavourable. Genetic resistance in plants to aboveground diseases is common, whilst genetic resistance to root diseases is rare (Cook *et al.*, 1995). According to these authors resistance to root pathogens primarily depends on the ability of the plant to support beneficial rhizosphere and endophytic microbial communities whilst suppressing deleterious ones. It is therefore not surprising that the literature contains many more examples of root disease biocontrol than of aboveground disease biocontrol.

For example, the phenomenon of 'induced suppression' is a natural one whereby a soil becomes suppressive to a soilborne pathogen during prolonged monoculture of the susceptible host. A suppressive soil can also be defined as one in which disease development is inhibited even when a pathogen is introduced in the presence of a susceptible host (Baker and Cook, 1974). Perhaps the most well known example of suppression is that of the wheat root disease 'take-all', caused by the fungus *Gaeumannomyces graminis* var. *tritici*, and the role of beneficial rhizobacteria in take-all decline that occurs in response to the disease and continued monoculture of wheat (Cook *et al.*, 1995). Although pathogen-suppressive soils are rare, those identified are excellent examples of the full potential of biological control of soilborne pathogens (Weller, 1988).

In this review we attempt to identify beneficial microorganisms and strategies from the literature which may be applicable for the improvement of sugarcane growth, primarily by improving root health, but also by the exclusion of deleterious microorganisms at planting.

Beneficial fungi

Trichoderma species are ubiquitous soil saprophytes. Many selected isolates are being employed as biological control agents to protect plants from fungal pathogens particularly *Rhizoctonia*, *Pythium* and *Fusarium* species attacking the roots. Among their other activities, *Trichoderma* isolates grow topically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade target fungal cell walls using combinations of proteases, cellulases, chitinases and glucanases (Inglis and Kawchuk, 2002). This process (mycoparasitism) limits growth and activity of plant pathogenic fungi. In addition to, or sometimes in conjunction with mycoparasitism, individual strains may produce antibiotics (Schirmbock *et al.*, 1994). There is also evidence for the triggering of host defense responses (Yedidia *et al.*, 1999), and for the promotion of growth in the absence of known pathogens or in sterilised soil. According to Altomare *et al.* (1999), the latter is due to the solubilisation of phosphates and micronutrients.

Roth (1971) noted that *Trichoderma* species were among the first fungal colonisers of filter cake, which can be used at sugarcane planting. Composted lignocellulosic organic materials such as filter cake are known to be colonised predominantly by *Trichoderma* and to be particularly suppressive to Oomycete pathogens (Kuter *et al.*, 1983). *Trichoderma* isolates from composted filter cake were capable of protecting sugarcane roots from attack by the Oomycete *Pythium arrhenomanes* (Roth, 1971).

Filter cake is also known to protect sugarcane roots from attack by nematodes when applied as a protective sheath (Cadet, 2002). A direct microbial effect on nematodes is also possible; for example, *Trichoderma* produces combinations of chitinases and proteases that may degrade the walls of nematode eggs (Mercer *et al.*, 1992). Sharon *et al.* (2001) evaluated *Trichoderma* for its

potential to control the root-knot nematode *Meloidogyne javanica*. All of the *Trichoderma* strains tested showed the ability to colonise *M. javanica* eggs and second-stage juveniles. One of the strains, genetically engineered for increased production of a particular protease, was even more effective.

There may also be an indirect microbial contribution towards nematode control *via* the control of root pathogens. In clover, *Pythium* infection of the roots has been shown to increase *Meloidogyne* nematode populations and the severity of root-knot symptoms (Zahid *et al.*, 2002), and in alfalfa, roots infected by *Fusarium oxysporum* were more attractive to *Pratylenchus penetrans* (Edmunds and Mai, 1967). Indeed, most of the reports of interactions between nematodes and root pathogenic fungi show that nematodes are favoured by the presence of the fungi (Faulkner and Skotland, 1965; Vrain, 1987; Hasan, 1988). The exclusion of pathogenic fungi from the roots should lead to a reduction in nematode parasitism.

Fusarium wilt of cotton, a disease of the water conducting vessels of the plant, is caused by the combined efforts of the fungus *Fusarium oxysporum* f.sp. *vasinfectum* and the root-knot nematode *Meloidogyne incognita* (Mai and Abawi, 1987). Symptoms of the disease can be much reduced by treating cottonseed at planting with preparations of nonpathogenic *F. oxysporum*. The nonpathogenic strain Fo47 forms an endophytic relationship with root epidermal and cortical cells from where it stimulates the plant to defend itself (Benhamou and Garand, 2001). Nonpathogenic strains are known to build up in soils becoming suppressive to wilt. However, the suppressiveness of such soils is not likely to be due to a single microbial population; fluorescent pseudomonads have also been implicated (Duijff *et al.*, 1999).

Protection by nonpathogenic strains is not unusual. A nonpathogenic mutant of *Colletotrichum magna* (path-1) has been shown to protect watermelon (*Citrullus lanatus*) and cucumber (*Cucumis sativus*) seedlings from anthracnose disease elicited by wild-type *C. magna* (Redman *et al.*, 1999). In doing so path-1 enters into a symptomless endophytic relationship with the plant. Disease protection in path-1-colonised plants is correlated with their ability to mount a defense response more rapidly, and to equal or greater levels, than plants exposed to wild-type *C. magna*. Path-1 either activates plant defenses to very low levels or primes the defense system without activation (Conrath *et al.*, 2002).

Obligate fungal endophytes

Whilst *Trichoderma* grows over the surface of the roots, and *F. oxysporum* Fo47 forms a superficial endophytic relationship, arbuscular mycorrhizal (AM) fungi have a more intimate obligate biotrophic relationship. They are an integral part of most plants in nature (Gianinazzi *et al.*, 1982) and occur on 83% of dicotyledonous and 79% of monocotyledonous plants investigated, including sugarcane (Wilcox, 1996). It has been well documented that mycorrhizal association benefits the host plant by increasing the uptake of nutrients, particularly phosphorous (P). An exchange is made for host carbohydrates. High soil P levels reduce mycorrhiza formation (Koide, 1991) and there is evidence that, under conditions of P deficiency, plants produce certain flavonoids that stimulate mycorrhizal formation (Akiyama *et al.*, 2002).

An important area of research is the use of mycorrhizae to prevent root disease, such as that caused by *Phytophthora* and *Pythium* species (Pozo *et al.*, 2002). There is no doubt that several mechanisms are at work, including a general strengthening of the plant through improved P nutrition. Mycorrhizae impose a physical barrier to pathogens, occupy niches, and may produce some protective antibiotics. The induction of plant defenses against pathogens has also been implicated (Cordier *et al.*, 1998; Shaul *et al.*, 1999). AM fungi have also been reported to limit plant parasitic nematode activity (Hussey and Roncadori, 1982), particularly if roots are colonised by the fungi before exposure to nematodes (Forge *et al.*, 2001).

AM fungi may promote beneficial and/or helper bacteria which may be directly responsible for protection against nematodes and pathogens, or for improved growth (Budi *et al.*, 1999). Free living *Burkholderia* species have been identified in the rhizosphere of plants and the hyphosphere of AM fungi (Andrade *et al.*, 1997). These bacteria are enriched in the total bacterial population. *Burkholderia* can also become endosymbionts of AM fungi and symbiotic nitrogen fixation can occur as a result (Bianciotto *et al.*, 2000).

Obligate non-pathogenic fungal-plant associations are not limited to AM fungi. In many grasses the fungus *Neotyphodium* forms a symptomless obligate biotrophic relationship, growing entirely within the host plant and being transmitted through the seed. These fungal endophytes have effects that are beneficial to the plant such as enhanced tillering, increased resistance to drought and insects, and the production of substances toxic to grazing animals (Bacon and Hill, 1996). Increased resistance to pathogens and nematodes has also been reported. This appears to involve induction of host resistance; for example, the presence of *Neotyphodium* elicits the production of chitinase by the plant (Roberts *et al.*, 1992).

Facultative fungal endophytes

Fusarium verticillioides is common as an endophyte of maize and it too forms a biotrophic association with the host plant (Bacon *et al.*, 2001). This association is usually symptomless although Pokkah boeng can result, similar to that found in sugarcane (Whittle and Irawan, 2000). However, in common with *Colletotrichum (Glomerella)*, the biotrophic phase can give way to a necrotrophic phase in which the fungus causes rots of stalks, ears and roots. The switch from biotrophy to necrotrophy may be brought about by damage, or changes in growing conditions. During both phases mycotoxins are produced which are toxic to mammals (Munkvold and Desjardins, 1997).

Other *Fusarium* species infecting maize include *F. subglutinans* and *F. proliferatum*. It is not known whether these species are capable of symptomless endophytic colonisation. Strains of *F. subglutinans* and *F. proliferatum* produce one or both of the insecticidal compounds beauvericin (Gupta *et al.*, 1991) and fusaproliferin (Munkvold *et al.*, 1998). Beauvericin is also produced by the entomopathogenic fungus *Beauveria bassiana* (Peczynska-Czoch *et al.*, 1991). Recently, *B. bassiana* has also been shown to be capable of forming an endophytic association with maize in which it does not lose its virulence towards the European Corn Borer, *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae) (Wagner and Lewis, 2000). It appears that *F. verticillioides* does not produce insecticidal toxins (Munkvold *et al.*, 1998).

In a survey in southern Benin, *F. verticillioides* was the most common endophytic fungus inhabiting maize stalks. Incidence was higher in plants damaged by insect pests, and it was cultured from stems of 71-80% of plants damaged by the stem borers *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Schulthess *et al.*, 2002). Insects feeding on infected plants may also vector the fungus (Sobek and Munkvold, 1999). It has also been found that ovipositing *E. saccharina* and *S. calamistis* moths not only prefer infected plants, but that offspring have higher survival and fecundity (Cardwell *et al.*, 2000). An effect on larval numbers was confirmed when it was shown that exclusion of *F. verticillioides* from maize seeds by hot-water-fungicide treatment reduced *E. saccharina* numbers in subsequent mature stalks and ears by approximately 50% (Schulthess *et al.*, 2002).

Bacterial epiphytes and endophytes

Rhizosphere bacteria are present in large numbers on the root surface, where plant exudates and lysates provide nutrients. Certain strains of rhizosphere bacteria stimulate plant growth, and are therefore often referred to as plant growth-promoting rhizobacteria (PGPR). Selected rhizobacterial strains, mainly fluorescent *Pseudomonas* and *Burkholderia* species, have been demonstrated to reduce plant diseases by suppressing soil-borne pathogens through competition for nutrients, siderophore-mediated competition for iron, or antibiosis (Schippers *et al.*, 1995). Plant parasitic nematodes can also be suppressed. Strains of *Burkholderia cepacia* and *B. gladioli* have been reported to be antagonistic towards soybean cyst and root-knot nematodes (Kloepper *et al.*, 1992) whilst a strain of the related bacterium, *Pseudomonas chloraphis*, is antagonistic towards root-lesion nematodes (Hackenberg *et al.*, 2000).

In addition to the mechanisms mentioned above, a form of induced disease resistance in the plant is triggered by non-pathogenic, root-colonising rhizobacteria. It is commonly referred to as rhizobacteria-mediated induced systemic resistance (ISR) (Van Loon *et al.*, 1998). Some strains are able to reduce disease through a plant-mediated mechanism that extends to the aboveground plant parts and is phenotypically similar to systemic acquired resistance (SAR) in being effective against different types of plant pathogens. Some rhizobacteria have been shown to trigger the SAR signalling pathway by producing salicylic acid at the root surface (De Meyer *et al.*, 1999). In other cases, ISR-inducing rhizobacteria have been demonstrated to trigger a different signalling pathway that does not require salicylic acid (Pieterse *et al.*, 1998). In those cases, bacterial determinants such as lipopolysaccharides from the outer membrane of *Pseudomonas fluorescens* have been shown to play an important role in eliciting ISR (Leeman *et al.*, 1995).

In general, endophytic bacteria originate from the epiphytic bacterial communities of the root or leaf surface. Souza (1996) obtained 189 endophytic bacterial isolates from three maize genotypes, at two localities in Brazil. The main genera observed were *Bacillus*, *Enterobacter*, *Leifsonia* (*Clavibacter*), *Pseudomonas* and *Pantoea* (*Erwinia*). Strains of *Bacillus subtilis* and *Enterobacter cloacae* have been shown to colonise maize stalks endophytically and to prevent subsequent colonisation by *Fusarium verticillioides* (Bacon *et al.*, 2001; Hinton and Bacon, 1995).

Leifsonia (*Clavibacter*) *xyli* subsp. *cynodontis*, a xylem-inhabiting coryneform bacterium that causes stunting disease in Bermuda grass, is capable of colonising several other plant species when artificially inoculated, including sugarcane, without causing disease (Bradbury, 1991). The closely related bacterium *L. xyli* subsp. *xyli* naturally infects the xylem of sugarcane, though in this case, ratoon-stunting disease (RSD) results (Davis and Bailey, 2000). The possibility that *L. x.* subsp. *cynodontis* can be introduced into sugarcane for the prevention of *L. x.* subsp. *xyli* infection has not yet been investigated. As well as the possibility of resistance priming by the non-pathogenic strain, closely related bacteria are known to inhibit each other through the production of peptide bacteriocin antibiotics (Gross and Vidaver, 1979).

Dominiak (1996) proposed the idea that there might be conducive soils for RSD. Unexplained transmission of *L. xyli* subsp. *xyli* was greatest in soils of high sand and low clay contents. Biddle *et al.* (1990) also note that Goss's wilt of maize, caused by *Clavibacter michiganense* subsp. *nebraskense*, is prevalent in maize grown on sandy soils in Nebraska. Nematodes are important constraints of sugarcane production on sandy soils in South Africa (Spaull and Cadet, 1990). Since endophytic bacteria, both pathogenic and non-pathogenic, usually require wounds for entry into the plant, it is possible that nematodes increase the infection rate of *Leifsonia* present in the soil, or present in root residues of the previous crop. The incidence of alfalfa bacterial wilt, caused by *Clavibacter m.* subsp. *insidiosum*, increases with nematode numbers (Hawn, 1971; Hunt *et al.*, 1971).

Implementation of plant health initiatives

It is of interest that genetic resistance in plants to root diseases is rare whilst genetic resistance to aboveground diseases is common. Agriculture depends instead on practices such as crop rotation to control root diseases (Cook *et al.*, 1995). However, growth and reproduction of the same plant species year after year on the same sites is typical of natural plant ecosystems. Pressure from soilborne pathogens may drive the selection of plants with the ability to support and respond to microorganisms capable of suppressing root pathogens, other deleterious rhizosphere microorganisms and nematodes. It might therefore be expected that there would be a significant effect of plant genotype on ability to support beneficial epiphytic and endophytic microbial communities. In *Arabidopsis* the ability to respond to ISR-inducing bacteria has recently been shown to be a heritable trait (Ton *et al.*, 2001).

In addition to selecting plant genotypes capable of supporting biocontrol microorganisms, the genotype of the biocontrol agent is also important. *P. fluorescens* Q8r1-96, is highly adapted to the rhizosphere of wheat where it limits the effect of wheat take-all. Q8r1-96 required a much lower inoculative dose and maintained a rhizosphere population density of approximately 10^5 colony-forming units per gram of root after eight successive crops in three different soils. The next best strain was not detectable after seven successive crops (Raaijmakers and Weller, 2001).

Another approach to improve disease suppression by biocontrol bacteria is the use of strain combinations. The strains to be combined can be selected for their modes of action, for example competition for iron, antibiosis and their involvement in ISR. Combinations of bacteria, biocontrol fungi and AM fungi have also been investigated. In this context, it has been found that certain *Pseudomonas* strains which produce antimicrobial compounds, and fungal biocontrol agents such as *Trichoderma*, do not inhibit AM fungi (Paulitz and Linderman, 1989; Calvet *et al.*, 1992; Barea *et al.*, 1998).

Examples of combinations include *Trichoderma koningii* with either of two biocontrol strains of *Pseudomonas chloraphis* or *P. fluorescens* providing greater suppression of wheat take-all than *T. koningii* alone (Duffy *et al.*, 1996). A strain of *T. (Gliocladium) virens* combined with *Burkholderia cepacia* was more effective than either alone in protecting maize roots from attack by a mixture of *Pythium ultimum*, *P. arrhenomanes* and *Fusarium graminearum* (Mao *et al.*, 1998). *T. auroviride* combined with the AM fungus *Glomus intraradices* enhanced the growth of citrus more than *G. intraradices* alone (Camprubi *et al.*, 1995).

The success of biocontrol seems to be greatly improved by the incorporation of specific types of organic matter that support the activities of the agents without stimulating the activity of the pathogens (Hoitink and Boehm, 1999). The use of filter cake at planting in sugarcane and the use of green manures are examples. A greater diversity of microorganisms occurs in soil as organic matter is increased, thus increasing the chances of microbial competition and antagonisms against soilborne pathogens (Baker and Cook, 1974). Alternate crops can also increase soil suppressiveness. Planting of velvet bean (*Mucuna deeringiana*) is associated with the development of an antagonistic microflora in soils and rhizospheres, generally increasing the frequency of *Bacillus* and *Arthrobacter* species, and *Burkholderia cepacia* (Vargas-Ayala *et al.*, 2000).

Improvement of biocontrol agents has also been attempted. Stasz *et al.* (1988) found that natural strains of *Trichoderma* were insufficiently effective as biocontrol agents. Protoplast fusion of selected wild strains introduced genetic diversity into progeny strains, a small percentage of which were of significantly improved biocontrol ability. One of these strains was also extremely rhizosphere competent; i.e. it colonised and protected the entire root system for the life of the crop. Moreover, it was effective against a wider range of plant pathogenic fungi. Attempts have also been

made to improve the efficacy of bacterial biocontrol agents by genetic engineering. For example, the ability to produce the antifungal agent phenazine-1-carboxylic acid was inserted into *Pseudomonas putida* WCS358r (Glandorf *et al.*, 2001) and an endophytic strain of *Pseudomonas fluorescens*, isolated from micropropagated apple plantlets, has been transformed with a chitinase gene from *Serratia marcescens* (Downing and Thomson, 2000).

Leifsonia xyli subsp. *cynodontis* has been developed as a biocontrol agent for lepidopterous stemborers through genetic engineering. Two copies of the *Bacillus thuringiensis* subsp. *kurstaki* cryIA(c) δ -endotoxin gene were integrated into the chromosome of *L. x.* subsp. *cynodontis* by Lampell *et al.* (1994). Transformed bacteria were inoculated into maize resulting in reduced damage caused by the European corn borer *Ostrinia nubilalis* (Tomasino *et al.*, 1995).

Concluding remarks

Cane production in the Umfolozi Mill area of the South African sugar industry is almost entirely on sandy soils of low organic matter content. About 30% of the area is infected by RSD, approximately double the average for the whole industry (McFarlane, unpublished results). If soils in the Umfolozi area are indeed conducive towards RSD, then suppressive soils might exist elsewhere. In the Midlands, the majority of soils are much higher in clay and organic matter contents than at Umfolozi, as well as being P-fixing and of lower pH. Only 4% of the area is infected by RSD. Baker and Cook (1974) state that a greater diversity of microorganisms occurs in soil as organic matter is increased, thus increasing the chances of microbial competition and antagonisms against soilborne pathogens.

Trichoderma and beneficial bacteria delivered in filter cake, or in other forms of organic matter, are being investigated for their suppressive effects on nematodes and pathogens infecting plant cane. This includes effects on the RSD bacterium, in so far as it can infect plant cane from the soil after the replacement of an infected crop (Bailey and Tough, 1992). During the composting process, the inoculation of filtercake with *Trichoderma* and bacterial strains of proven biocontrol and rhizosphere competence might be attempted.

The Pathology Department at SASEX has begun research on AM fungi in which isolates are tested for their ability to protect sugarcane roots from nematode and pathogen attack. Their influence on P nutrition and contribution towards resistance to aluminium toxicity, particularly in P-fixing Midlands soils, is also being investigated. It is expected that the colonisation of sugarcane roots by mycorrhizal fungi will take place to a greater degree in P-fixing soils than in non-P-fixing soils.

A finding of particular significance to South African sugarcane production was that the exclusion of *F. verticillioides* from maize seeds by hot-water-fungicide treatment reduced *E. saccharina* numbers in subsequent mature stalks and ears by approximately 50% (Schulthess *et al.*, 2002). A preliminary investigation into the occurrence of endophytic *Fusarium* in sugarcane suggests that a similar interaction between the fungus and *E. saccharina* may be taking place (unpublished results). A joint project has been initiated between the Pathology and Entomology Departments at SASEX to investigate the interaction between sugarcane, *E. saccharina* and *Fusarium* species.

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