

BIOCONTROL OF PLANT DISEASES - POSSIBLE BIOCONTROL APPROACHES AGAINST WHITE ROOT DISEASE OF RUBBER

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Non degradable residues of chemical pesticides can have a major impact on the environment. Pesticides which are readily metabolized or having broad spectrum of activity can endanger non-target pests or other components of the ecosystem. Therefore, the importance of biological control of pests and disease stands un-controversial. The term 'biological control' was defined by Debach (1964) as 'the action of predators, parasites or pathogens in maintaining another organisms population density at a lower average than would occur in their absence'. Similarly, several attempts have been made to define the term biological control in different views. Papavizas and Lumsden (1980) described the same as the reduction of the propagule number of the pathogen or disease producing ability of those propagules to a tolerable or economically acceptable level'.

It is accepted that by adopting biological control methods it may be possible to avoid development of new chemical resistant pathogenic strains and maintain relatively problem- and risk-free control. The problem of developing pesticide resistant strains is not yet as serious in plant pathology as in entomology (Smith, 1972). Nevertheless, populations of plant pathogens with resistance to chemicals used for the control are known, including resistance to benzimidazole fungicides by *Venturia inaequalis* (cause of apple scab), *Erysiphe cichoracearum* (Powdery mildew of cucurbits), *Botrytis cinerea* (on several plants), resistance to metalaxyl by *Phytophthora infestance* (late blight of potato), *P. parasitica* var *nicotiana* (Black shank of tobacco) and *Perenespora tabacina*. (tobacco blue mold). In general, biological control may be accomplished through a) cultural practices that create an environment favourable to antagonists, b) plant breeding to improve resistance to the pathogen or the suitability of a host plant to the activities of antagonists, or c) the mass introduction of antagonists, non-pathogenic strains or other beneficial organisms (Lewis & Papavizas, 1991).

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Major constrains to biocontrol

The use of biological antagonists to control plant disease is limited. The complex microbial community in soils imposes a natural buffering effect on population increase by individual species which is equivalent to the general

suppressiveness of soil and operates against newly introduced biocontrol agents (BCAs). Therefore, BCAs are reported to be effective when targeting to specific sites on plants that favor their activities or applied to soils that are particularly receptive so that the BCA can proliferate (Deacon, 1991). As biocontrol involves living organisms, their production, storage and application might be a problem. The type of BCA will have the critical effect on manufacturing and formulation. A major problem in production is the lack of a large arsenal of highly effective, stable, long-lived organisms and the questionable adaptability of such organisms to industrial processing. Methods of application to soil or any specific sites would depend on the processed form of BCA and simultaneously there will be unknown health and environmental risks from the use of most experimental BCA. Determination of type of BCA to control a disease should be based on the screening process. The selection of the appropriate growth stage of BCA is also important for effective control. If spores is required, then the process should be adjusted accordingly.

Large quantities of inocula are required to control some diseases. To control diseases with *Trichoderma* species one needs a soil population of those of at least 10^5 propagules per g soil (Adams, 1990). When refer to the production of BCA's under controlled conditions, production of large quantities may be a difficult task.

The release of the BCA to environment should be made under the control of regulating agencies. Problem of testing of genetically modified organisms (GMOs) in many countries stand controversial because of the risk of entering foreign DNA to the environment. Apart from these constraints, the response of the farming community about safety and efficacy would be doubtful. Hence, the education of the farming community would be necessary.

Mechanisms involved in biocontrol

Antagonism

The fungi existing in a particular habitat such as soil or the surface of a plant organ, generally are in ecological balance with other micro-organisms around them, perhaps, through natural competition. But it may be possible to add one organism that harm most others or curtails their activities by releasing some toxic compounds. This phenomenon could be used as an important approach to the manipulation of fungi as BCAs. In nature, many *Trichoderma* and *Glicocladium* species are known to be antagonistic to several plant pathogens (Chet, 1987; Papavizas, 1985). Antagonism is highly correlated with availability of nutrients and other resources in the rhizosphere. If one organism is weaker than others to compete for available resources, then it will be inefficient for persistence in competitive rhizospheres such as soil. The initial number of propagules might be an important factor in this respect.

Therefore, in successful biological control, the augmentation of a new antagonist in a suitable pre-colonized nutrient substrate can be beneficial. In nature, antagonistic organism can operate by rapid colonization in advance of other organism or subsequent competition or combat. In competition of different organisms, antibiotics may be produced or there may be mycoparasitism or the lysis of one organism by others.

Antibiosis

Antibiotics are generally considered to be organic compounds of low molecular weight, produced by microbes. These compounds are deleterious to the growth or metabolic activities of other organisms at low concentrations (Fravel, 1988). They are produced only in rich substrates for example, carbohydrate rich substrate where the competition is extreme. Such antibiotics can reach other organisms through diffusion in water, or air (Faull, 1990). Those which are tolerable to antibiotics in their habitats would survive in such instances. In addition, fungi such as *Trichoderma* and *Gliocladium* spp. release volatile antibiotic compounds which inhibit some pathogenic fungi *in vitro* (Dennis & Webster, 1971).

Mycoparasitism (In fungi)

For plant disease control some of the most intensively investigated organisms have been mycoparasites, *i.e.* fungi that parasitise other fungi, growing at the expense of nutrients from a fungal host (Deacon, 1976). According to Chet *et al.* (1981), mycoparasitism is a complex process including several steps: growth of hyphae of a mycoparasite directly towards its host, under the influence of a gradient of chemical excreted by the host (Chet & Elad, 1983), coiling of mycoparasite hyphae around the host hyphae or attachment to them by forming hook-like structures (*eg. Trichoderma hamatum* can produce appressoria at the tips of short branches), and, following these interactions, mycoparasites may penetrate the host mycelium by partially degrading its cell wall (Elad *et al.*, 1983a,b). Proteases, glucanases and cellulases are reported to be enzymes involved in the action of cell wall degrading in pathogenic fungi by *Trichoderma* spp. Elad *et al.*, 1982; Sivan & Chet, 1988).

The order Mucorales contains parasites of many mucorales, but these hosts are not significant as plant pathogens. Many ascomycetes, fungi imperfect and some higher basidiomycetes are also known to be mycoparasites (Barnett, 1963). Of more relevance are fungi that parasitise ascomycetes, basidiomycetes and imperfect fungi. Depending on the methods of nutrient uptake from the host, mycoparasites have been divided into 1) balanced or biotrophic mycoparasites and 2) necrotrophic or destructive mycoparasites (Barnett & Binder, 1973).

There are a number of examples of fungi that parasitise plant pathogens Lumsden (1981); Barnett & Binder (1973) and Cook & Baker (1983) listed attributes of at least 24 agents that potentially can suppress plant diseases. According to many authors, *Trichoderma* is a mycoparasite with much potential for successful biological control (Boosalis, 1964; Hader *et al.*, 1979; Harman *et al.*, 1980; Chet & Baker 1980; Elad, *et al.*, 1983b).

Predation

Predacious fungi occur in divers taxonomic groups such as order of zoopagales of the zygomycetes and also in the family moniliaceae of fungi imperfecti. Various techniques of prey capture are employed by different fungi such as *Dactylella ellipsozona* (adhesive knobs), *Arthrobotrys oligospora* (adhesive hyphal networks) and *Dactylella lysipage* and *A. dactyloides* (constructing rings) (Burge, 1988). In some species a toxin is produced that immobilizes the nematode instantly, followed by hyphal penetration through the cuticle (Mankau, 1980). The interactions between nematode-trapping fungi and nematode is reported to be complex. Soil pH, moisture, temperature and the availability of nutrients can be controlling factors of these fungi (Johnsan, 1962; Mankau, 1968). Trapping fungi are non-specific producing traps having ability to capture a range of nematode species with equal efficiency (Kerry, 1984).

Approaches on mass production of biocontrol agents

Several attempts have been made to develop profitable methods on mass production of BCA. Lewis and Papavizas (1984) tested a preparation consisting of 1% bran and $10^3 - 10^4$ young actively growing propagules per gram soil. Papavizas *et al.* (1984) improved another technique of this nature where they used a liquid media consisting of molasses and brewers yeast. The maximum biomass was achieved from *Trichoderma viride* after 10 days and from *Gliocladium virens* after 15 days of agitative incubation. In addition, some authors such as Lewis *et al.* (1991), described a new formulated system that does not require sterile conditions during preparation. These gradual improvements eliminates the future prospects of the practical approaches on application of biocontrol techniques in modern agriculture.

Biocontrol successes in commercial level

There are many examples of commercial exploitation of BCA on disease control. Among those, control of *Heterobasidion annosum* root disease of pine by *Peniophora gigantea* (*Phlebiopsis gigantea*) was successful. Rishbeth (1963) reported the possible use of oidiospores of *Peniophora gigantea* (*Phlebiopsis gigantea*) for inoculation of newly felled pine stumps applying on the cut surface against the pathogen. The product on commercial level is registered as PG. Similarly, strains 84 of *Agrobacterium radiobacter* was found to be non-pathogenic and produced antibiotics against *A. radiobacter* var. *tumefaciens* which cause crown gall of citrus and fruit trees (Kerr, 1980). *Pseudomonas fluorescens* developed by Ecogen, Inc. of Langhorne, PA, USA is sold as Dagger G* for use against *Rhizoctonia* and *Pythium* damping-off of cotton. *Gliocladium virens*, developed by the USDA-ARS group at Beltsville, MD is sold as GlioGard to use against seedling diseases of ornamental and bedding plants (Lumsden & Walters, 1993). The fungus is produced in an alginate-wheat bran formulation (Whipps & Lumsden, 1991). *Trichoderma harzianum* developed at the Cornell University at Geneva is registered by Kodak as F-Stop* (Cook, 1993). *Trichoderma harzianum/polysporum* known under the trade name of BINAB T for use against Wood decay (Ricard, 1981). In countries such as USA or in Europe, microorganisms intended for pest control, if introduced on more than 10 acres (3.9 ha) require an Experimental Use Permit (EUP), and they must be registered as a 'microbial pesticide' before they can be sold. Most of the above microorganisms are registered in the United states for use against plant diseases (Cook, 1993).

Genetically improved strains of antagonists developed through genetic manipulation should also be noted in this context. An improved *Agrobacterium radiobacter* K-84 namely strain K-1026 produced through a DNA deletion is the first genetically manipulated antagonist used commercially. From this modification, the breakdown of biological control of crown gall by the agrocin-producing strain of avirulent *A. radiobacter* is retarded or prevented, and thereby prolonged the utility of this biocontrol (Cook, 1993).

Possible biocontrol approaches against white root disease of rubber

At present, number of chemicals including Tridemenol (Bayfidan) and Tridemephone (Bayleton) show effectiveness in controlling the fungus *in vitro* conditions. Similarly, successful results were also obtained using a) chemicals of the triazole group (Jollands, 1983; Tran, 1985; Tan, 1990; Ng & Yap, 1990; Gohet *et al.*, 1991), b) 2% Pentachlorophenol in bituminous base (Jayasinghe *et al.*, 1995), c) biological control agents (Tong-Kwee & Keng, 1990; Jayasuriya & Deacon, 1995),

d) fumigants such as furfuraldehyde (Jayasuriya & Deacon, 1996; Jayasuriya *et al.*, 1996).

However, eradication of a pathogen population with high dosages of killing agents involve detrimental effects indicated in a previous para. This situation is highly related to soil-borne pathogens such as *R. lignosus* which are deeply embedded in the soil. An alternative means of control would be to use sublethal dosages which may weaken the pathogen and enable reasonable and effective control while minimizing the above difficulties (Katan *et al.*, 1992). Thus, the weakened pathogen population would be replaced by competitive antagonists such as *Trichoderma* species. It was an adopted technique used in biocontrol of *Armillaria mellea*. (Ohr *et al.*, 1973). Work of Munnecke *et al.* (1976) revealed the effect of heat treatment on weakening of *A. mellea* infected roots after which the dominant colonizer was identified as *Trichoderma* species. Several other successful results have also been obtained for pathogens such as *Sclerotium rolfsii* (Smith, 1972).

However, it is recognized that biocontrol can seldom give complete disease control (Chet, 1987). Nevertheless, it might be possible to modify the cropping environment to favor the activities of resident antagonists and simultaneously suppress the pathogen. Even sub-lethal rates of application of fungicides, or sub-lethal physical treatments such as soil solarization (Katan *et al.*, 1992) can be effective for this, because pathogen inoculum that is weakened is more susceptible to antagonism or replacement by resident antagonists. There are many practical and experimental examples for a control of this nature (Garrett, 1957; Ohr *et al.*, 1973; Elad *et al.*, 1981a,b).

On controlling *H. annosum* root disease of pine, by *P. gigantea*, the mode of action of *P. gigantea* on *H. annosum* was subsequently found as contact mediated inhibition termed "hyphal interference" (Ikediugwu, 1970; Ikediugwu & Webster 1970 a,b; Ikediugwu, *et al.*, 1970). The mechanism remains largely unknown. Ikediugwu, *et al.* (1970) found no evidence that *Phlebiopsis* gained directly from this antagonism, *i.e.* no evidence that it acts as a mycoparasite. Instead, hyphal interference was suggested to be a mechanism in which *Phlebiopsis* can antagonise other fungi that are potential competitors for the same underlying substrate.

As *R. lignosus*, is also a basidiomycete, it might be controlled by other basidiomycete that cause hyphal interference. But this basidiomycete should not be a pathogen to the rubber tree. Jayasuriya & Deacon (1995) studied *in vitro* antagonistic activities of some potentially antagonistic fungi against *R. lignosus* in which they have isolated *Trichoderma* and *Gliocladium* species which are highly antagonistic. In addition, they also reported that species of *Trametes* and *Schizophillum commune* are having antagonistic properties against *R. lignosus in vitro*. In field conditions, *R. lignosus* can grow through soil as mycelial aggregates (cords) from food base such as diseased roots of standing trees or major woody crop residues

in soil, and infect further trees from these mycelial cords. So, one approach to biocontrol (or integrated control) is to prevent or reduce the spread of mycelial cords through soil. This might be achieved by making the soil inhibitory to the pathogen by physical, chemical or biological manipulation. Additionally, it might be possible to use sub-lethal treatments to weaken the pathogen on its food base, so that it soon replaced by antagonistic microorganisms. This is an established method of biocontrol of fungi that invade tree roots, but the fumigants used to date are extremely hazardous (e.g. methyl bromide). Hence it is utmost important to find out a suitable mild fumigant to be used in this respect. Studies of Jayasuriya & Deacon (1996) and Jayasuriya *et al.* (1996) showed the potentiality of furfuraldehyde to be used as a mild fumigant in controlling *R. lignosus*.

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