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Review

Biological control of plant diseases: the European situation

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Abstract

The most common approach to biological control consists of selecting antagonistic microorganisms, studying their modes of action and developing a biological control product. Despite progress made in the knowledge of the modes of action of these biological control agents (BCAs), practical application often fails to control disease in the fields. One of the reasons explaining this failure is that the bio-control product is used the same way as a chemical product. Being biological these products have to be applied in accordance with their ecological requirements. Another approach consists of induction of plant defence reactions. This can be done by application of natural substances produced by or extracted from microorganisms, plants, or algae. Since they do not aim at killing the pathogens, these methods of disease control are totally different from chemical control. Although promising, these methods have not been sufficiently implemented under field conditions. A third approach consists of choosing cultural practices that might decrease the incidence or severity of diseases. These methods include the choice of an appropriate crop rotation with management of the crop residues, application of organic amendments and the use of new technology such as the biological disinfestation of soils. Biological control practices need an integrative approach, and more knowledge than chemical control.

Introduction

Biological control is a strategy that was proposed half a century ago. Indeed, a symposium held in Berkeley in 1965 was entitled: 'Ecology of soil-borne plant pathogens; prelude to biological control' (Baker and Snyder, 1965). Forty years later, biological control of plant diseases is still in its infancy, and only a few biological control agents (BCAs) are available on the European market. At the same time there is a demand from society for healthy foods with less chemical residues, and a great concern for preservation of the environment. It is therefore interesting to review progress and failures in biological control research and their implementation to avoid repeating the same mistakes as in the past.

Many different definitions of biological control have been proposed. Before reviewing biological control practices, it is important to define these terms. According to Cook and Baker (1983) "Biological control is 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". Based on this broad definition of biological control, organisms and procedures involved include: (i) avirulent or hypo-virulent individuals or populations within the pathogenic species, (2) antagonistic microorganisms, and (3) manipulation of the host plant to resist the pathogen more effectively. Biological control may be accomplished through several approaches including mass introduction of antagonists, plant breeding, and specific cultural practices aimed at modifying the microbial balance.

In this review we will consider not only microbiological control, which is the use of populations of specific BCAs, but also cultural practices which contribute to decreasing disease incidence or disease severity. We will not present strategies of plant breeding or plant transformation for resistance, which deserve full reviews by themselves, but we will discuss the strategies used to induce systemic resistance within the plant using microorganisms or natural elicitors. Although the principles of biological control are similar when considering aerial diseases or soil-borne plant pathogens, most of the examples presented below will be taken from our own field of interest which is biological control of soil-borne diseases.

Microbiological control

During the three last decades there has been a trend to screen for effective antagonists and to develop them as BCAs. Biological control has been often limited to massive applications of products containing a BCA. In contrast to the many published papers dealing with the modes of action of the antagonists, there are only a few papers dealing with application of the bio-control products. Obviously, before developing a product based on an antagonistic microorganism, it is necessary to understand its modes of action, but production, formulation and strategies of application also need to be carefully addressed (Burges, 1998). Antagonistic effects responsible for disease suppression results either from microbial interactions directed against the pathogen, mainly during its saprophytic phase, or from an indirect action through induced resistance of the host plant.

Microbial antagonism

Microbial antagonism implies direct interaction between two microorganisms sharing the same ecological niche. Three main types of direct interactions may be characterised: parasitism, antibiosis and competition for nutrients.

Parasitism of a plant pathogen by other microorganisms, including viruses, is a well known phenomenon. The parasitic activity of strains of *Trichoderma* spp. towards pathogens such as *Rhizoctonia solani* has been extensively studied (Chet and Baker, 1981). It involves specific recognition between the antagonist and its target pathogen and several types of cell wall degrading enzymes to enable the parasite to enter the hyphae of the pathogen. Other mycoparasites such as *Coniothyrium minitans* (Jones et al., 2004a) and *Sporidesmium sclerotivorum* (Adams and Fravel, 1993) are effective in controlling diseases caused by *Sclerotinia* spp. and other sclerotia-forming fungi. Two strains of *Coniothyrium minitans* have been developed as biological control products and are available on the European market. This type of antagonism, which causes the death of the target organism results in a decrease of inoculum density. However, parasitism of fungal pathogens by viruses or virus-like particles such as dsRNA induces hypovirulence. Hypovirulent strains of *Cryphonectria parasitica* are used in practice to control chestnut blight. Hypovirulence is contagious, since dsRNAs can be transmitted from a hypovirulent strain to a virulent compatible strain, and under favourable conditions hypovirulence can spread naturally in diseased orchards (Milgroom and Cortesi, 2004).

Antibiosis is the antagonism resulting from the production by one microorganism of secondary metabolites toxic to other microorganisms. Antibiosis is a very common phenomenon responsible for the activity of many BCAs such as fluorescent *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp. and *Trichoderma* spp. A large diversity of molecules has been described and their role in suppression of several plant pathogens has been documented (Fravel, 1988; Loper and Lindow, 1993; Weller and Thomashow, 1993). They include not only antibiotics *sensu stricto*, but also bacteriocines, enzymes such as cell wall degrading enzymes, and volatile compounds with an antifungal activity. A given strain of a BCA may produce several types of secondary metabolites having different functions and effective against different species of fungal pathogens. For example, the strain CHAO of *Pseudomonas fluorescens* produces siderophores, phenazines, 2,4-diacetylphloroglucinol and cyanide, a different combination of these metabolites being responsible for the antagonism expressed against *Gaeumannomyces graminis* var. *tritici* and *Chalara elegans* (Défago and Haas, 1990). To clearly establish the role of these secondary metabolites in the activity of a given strain of a BCA one must use biotechnological tools enabling the production of mutants that are deficient for the production of a given molecule. Such an approach was proposed for the first time by Thomashow and Weller (1988) who demonstrated the role of phenazine in the biocontrol activity of a strain of *Pseudomonas fluorescens* against *Gaeummanomyces graminis* var. *tritici*. Strains of *Trichoderma* spp. produced many types of secondary metabolites (Sivasithamparam and Ghisalberti, 1998) including antibiotics (Howell, 1998) and cell wall degrading enzymes (Kubicek and Penttilä, 1998; Lorito, 1998) whose roles in biocontrol activity have been clearly established.

It is important to emphasise that a single antifungal metabolite generally does not account for all the antagonistic activity of a BCA. Different secondary metabolites produced by a strain of a BCA might be responsible for the antagonistic activity towards different pathogens, as illustrated above with the example of the strain CHAO of *Pseudomonas fluorescens*. Therefore one must avoid any generalization from one patho-system to another patho-system, and from one BCA to another BCA.

Competition for nutrients is a general phenomenon regulating population dynamics of microorganisms sharing the same ecological niche and having the same physiological requirements when the trophic resources are limited. Competition for nutrients occurs at the leaf surface where it can prevent the germination of fungal spores and reduce infection (Fokkema, 1996). Competition for nutrients, especially for carbon, is common in soil which is an oligotrophic milieu; it is considered responsible for the well-known phenomenon of fungistasis (Lockwood, 1977) describing the inhibition of fungal spore germination in soil. Although difficult to demonstrate rigorously, competition for nutrients is certainly one of the modes of action of many BCAs.

One of the best documented examples concerns competition for carbon between pathogenic and non-pathogenic *Fusarium oxysporum*. Having demonstrated that competition for carbon on one hand and populations of non-pathogenic *Fusarium oxysporum* on the other hand were involved in the mechanisms of soil suppressiveness to Fusarium wilts, it was hypothesised that competition for nutrients was one mode of action of non-pathogenic *F. oxysporum*. Evidence that competition is one mode of action of BCAs is provided by study of quantitative interactions between the pathogen and the BCA. Determining the growth parameters of strains of *F. oxysporum* competing for the colonization of disinfected soil, Couteaudier and Alabouvette (1990) established that some strains of non-pathogenic *F. oxysporum* were more competitive than others and should be selected for biological control.

Competition for minor elements also frequently occurs in soil. For example, competition for iron is one of the modes of action by which fluorescent pseudomonads limit the growth of pathogenic fungi and reduce disease incidence or severity (Schippers et al., 1987; Bakker et al., 1991; Lemanceau and Alabouvette, 1993; Loper and Henkels, 1997). Competition also occurs on root surfaces. Mandeel and Baker (1991), studying interactions between pathogenic and non-pathogenic *F. oxysporum*, postulated that the root surface had a finite number of infection sites that could be protected by increasing the inoculum density of the non-pathogenic strain. Only recently, the use of strains expressing different reporter genes enabled the visualization of interactions between pathogens and BCAs at the root surface. Bolwerk et al. (2003) showed that in the presence of fluorescent pseudomonads the fungal growth was restricted at the surface of the tomato root. Following the same approach, Olivain et al. (2006) observed intense colonization of the surface of tomato roots by a strain of non-pathogenic *F. oxysporum*, that competed with a strain of *F. oxysporum* f.sp. *lycopersici*. Depending on the respective concentration of the two fungi, the growth of the pathogen could be greatly restricted, but never totally suppressed. Although very common, competition between BCAs and pathogens is a phenomenon difficult to exploit for biological control.

Systemic induced resistance (SIR)

Any plant reacts to stresses from biotic or abiotic origin by elicitation of defence reactions. The plant reacts to: (i) physical stresses such as heat or frost, (ii) inoculation by pathogenic or non-pathogenic organisms, (iii) chemical molecules from natural or synthetic origins, by expressing defence reactions leading to systemic induced resistance (SIR). This phenomenon has been intensively studied at the cellular and molecular levels. Elicitation of plant defence reactions involves an early recognition of the aggressor by the plant. This molecular recognition immediately initiates a cascade of molecular signals and the transcription of many genes. This results in the production of defence molecules such as phytoalexins, pathogenesis-related (PR) proteins and reinforcement of cell walls (Van Loon, 2000). The build up of barriers such as cell wall thickenings and wall appositions contributes to slowing down the progression of the fungus. A specific and well-documented case of SIR is that of the hypersensitive reaction induced by a virulent pathogen on a resistant plant cultivar. Among other reactions, the hypersensitive response results in the death of the infected cells; this stops the development of the biotrophic pathogens.

SIR classically occurs when an inducing agent, pathogenic or not, is applied prior to challenge inoculation with a pathogen, resulting in reduced disease in comparison to the non-induced control. Kuc (1987) reported the first evidence of systemic protection of cucumber against *Colletotricum orbiculare* after pre-inoculation of the cotyledons of the plant with the same pathogen. It is also well established that the pre-inoculation of a host-plant with an incompatible *forma specialis* of *F. oxysporum* results in reduced disease severity when the plant is inoculated with the compatible pathogen (Biles and Martyn, 1989). More and more studies are devoted to SIR in the host plant after application of biological control agents. Where soilborne pathogens are concerned, papers report experiments where a non-pathogenic strain applied to some roots of a host plant can delay symptom expression induced by the pathogen when separately applied to other roots (split-root system) or directly into the stem of the plant (Biles and Martyn, 1989; Mandeel and Baker, 1991; Kroon et al., 1992; Olivain et al., 1995; Fuchs et al., 1997; Larkin and Fravel, 1999). Since there is no direct interaction between the two microorganisms, the observed disease reduction is attributed to increased plant defence reactions in response to root colonization by the non-pathogenic strain. Fuchs et al. (1997) attributed the biocontrol activity of the non-pathogenic strain Fo47 to SIR in tomato, correlated with an increased activity of chitinase, ß 1-3 glucanase and ß 1-4 glucosidase. More recently, Olivain et al. (2003) characterized the early physiological plant defence reactions in response to inoculation with germinated conidia of pathogenic and non-pathogenic strains of *F. oxysporum*. They showed that the non-pathogenic strain induced in flax cells some of the physiological reactions (increased pH, hydrogen peroxide production, Ca^{2+} influx, and cell death) associated with SIR in other plant-pathogen systems.

Many BCAs have been reported to induce systemic resistance in plants. This is the case for the fluorescent pseudomonads selected for their plant growth promoting capacity (Kloepper et al., 1993; Van Loon et al., 1998) and for *Trichoderma* species (Harman et al., 2004b). Since SIR is a general phenomenon that can protect the plant against several pathogens it sustains more attention today that any other mode of action of BCAs. However, it must be said that SIR and other modes of action are not mutually exclusive; SIR might only exert a complementary effect to microbial antagonism.

Advantages and limits of microbiological control

Despite the many research efforts dealing with biological control of plant diseases, application of microbiological control is limited. Today there are only four strains of BCAs (*Ampelomyces quisqualis, Coniothyrium minitans, Paecilomyces fumosoroseus, Pseudomonas chlororaphis*) on Annex I of EEC directive 91/414, that is the list of strains that may be used in the European Union. Even if we add the two dozen products which were registered in different European countries before application of the directive, there is still only a limited number of products available for growers. The use of microbiological products to control plant diseases is more common in some other countries, especially the USA, Australia and New Zealand (see below).

The main criticism made of microbiological control is the lack of consistency. Several reasons can account for this. One claimed advantage of biological control is the narrow specificity of the BCAs. Their application will not affect the non-target organisms and therefore will respect the environment better than large spectrum molecules. But in practice, the population of a pathogenic organism presents a certain diversity and a single given strain of a BCA might not have the same efficacy on all the pathotypes present in the population (Schisler et al., 2000). Moreover, the climatic conditions most favourable for the development of the pathogen might not be the same as the conditions required for maximum expression of the antagonistic activities of the BCA (Nicot et al., 2002). It is necessary to carefully study the effect of inoculum type, application rate and time of application to ensure efficacy of biological control (Jones et al. 2004a, b). Finally, both the diversity of the natural population of the pathogen and the climatic conditions in nature are much more variable than those used in the laboratory to study the modes of actions of a BCA. Other constraints result from the diverse modes of action of the BCAs which have different consequences on the population density and activity of the target pathogens.

When antagonistic activity results in a decrease of the inoculum density, as for example with the parasitism of *Coniothyrium* on *Sclerotinia sclerotiorum*, the practical efficacy directly depends on the quantitative relationship between inoculum density and disease severity. When ascospores produced by apothecia formed by sclerotia are responsible for plant infection, even a 95% reduction in the number of surviving sclerotia might not be sufficient to significantly reduce the infection rate of the plant.

To be effective, a BCA with a mode of action of antibiosis has to be applied at the right place and the right time. Indeed the secondary metabolites are not produced in great quantities and might not be transported a great distance. Thus it is necessary to place the antagonist in the infection court to be certain of contacting the pathogen. One example is that of *Pseudomonas chlororaphis* used as a seed dressing to control some soil-borne diseases of barley. This strain is effective through the production of 2,3-deepoxy-2,3-didehydro-rhizoxin (DDR) but does not actively colonize the rhizosphere of the plant. However, it is effective against fungi which infect young seedlings because there is a good spatial and temporal coincidence between the activity of the pathogen and activity of the BCA.

When competition is the main mode of action, the population of the biocontrol fungus must be at least as large, if not larger, than that of the pathogen population in order to achieve control. This can be done easily in laboratory experiments but is much more difficult to reach under field conditions where the inoculum density of the pathogen is not known. Non-pathogenic *F. oxysporum* provides a good example to illustrate the importance of the mode of action in relation to application dose. The strain Fo47, which functions mainly through competition, is only effective when it is introduced at concentrations 10–100 times higher than the pathogen concentration, whereas strain CS-20, which incites induced resistance, can significantly reduce wilt incidence in tomato when the pathogen population is up to 1000 times greater than that of strain CS-20 (Larkin and Fravel, 1999).

Thus, theoretically the most interesting mode of action should be SIR. Indeed, after elicitation, the resistance will be expressed in several if not all plant tissues and at a great distance from the point of inoculation of the BCA. But in practice, it is not so clear. One must remember than in nature a plant is permanently submitted to stresses of different origins. Application of a microbiological control agent might add some degree of resistance to the already expressed level of resistance, but this might not be enough to fully protect the plant. Moreover, as clearly demonstrated with several models, plant reaction depends on the plant species and even on the plant cultivar, on the physiological stage of the plant and on the environmental conditions (Van Loon, 2000).

It is our opinion that in order to make microbiological control more reliable, much more research should be devoted to the conditions required for the expression of the beneficial effects of BCAs under field conditions. Unfortunately, this type of field-oriented research is not supported by research institutions, and many potentially interesting BCAs have never been tested under field conditions.

Association of several modes of action

To improve consistency of biological control it would be useful to associate several modes of action, expressed simultaneously or successively. As stated above, most of the effective BCAs possess several modes of action and may be responsible for the control of different diseases on the same host or the same disease on several hosts (*Pseudomonas* spp or *Trichoderma* spp). Thus, one can expect a better efficacy and/or a better consistency if complementary modes of action are expressed simultaneously. Two ways have been explored to associate several modes of action in the same product: associations of several BCAs or association of several modes of action in the same strain of a BCA.

Lemanceau and Alabouvette (1991) were the first to propose the association of a strain of non-pathogenic *F. oxysporum* with a strain of *Pseudomonas fluorescens* to control Fusarium wilts. They had previously demonstrated that competition for iron created by *P. fluorescens* producing siderophores reinforced competition for carbon between the pathogenic and the non-pathogenic strains of *F. oxysporum* (Lemanceau et al., 1992). The control of Fusarium wilt by the non-pathogenic strain Fo47 was always improved by the association of a strain of *P. fluorescens*. The efficacy of the control depended on the concentration of the non-pathogenic strain Fo47 since the beneficial effect of the *Pseudomonas* strain was not dose-dependant (Olivain et al., 2004).

The second approach consists of the association of several modes of action in a single antagonistic strain, by genetic manipulation. The first improved strains of *P. fluorescens* producing phenazine and phloroglucinol have been evaluated for their improved biological control activity under field conditions (Thomashow and Weller, 1996). The association of several modes of action has also been proposed to enlarge the activity spectrum of BCAs. Finally, association of several strains having different ecological requirements might improve the 'ecological fitness' of BCAs and thus improve the consistency of biological control under variable environmental conditions.

Use of natural products to stimulate plant defence reactions

As stated above, plant resistance can be induced by stresses of different origins and, currently, there is a trend to study the effects of 'natural products' to induce systemic resistance in plants. There is a great diversity of natural products that can be used as elicitors of plant defense reactions: they include plant extracts such as neem oil, algal extracts such as laminarin, secondary metabolites produced by microorganisms such as harpin, essential oils such as geraniol or menthol, etc. Some of these products have been used traditionally in certain countries such as neem extracts in Asia, whilst others are novel extracted products, such as laminarin which was recently put on the market in Europe. The basic mechanisms by which the plant reacts after elicitation of its defence mechanisms by natural products are the same as those cited above in the case of elicitation by antagonistic microorganisms.

It might be questioned if the use of such chemical molecules should be considered as biological control. There are two arguments in favour of these molecules. They are of natural origin, and their mode of action targets not the pathogen itself, but the reinforcement of plant defence reactions. For us, the first argument has no scientific value since a molecule from natural origin does not differ from the same molecule obtained by chemical synthesis. But the natural origin of the molecule is required by organic farmers. The efficacy of these natural products is dependent on the same factors and conditions as those which control the efficacy of antagonists working through induced resistance: plant species and plant cultivar, physiological stage of the plant, inoculum pressure, and climatic conditions, to name a few.

A positive selling point for these natural products is that, in some countries, they are not considered plant protection products but 'plant strengtheners' and thus escape the regulations applying to plant protection products. According to European Regulations, if their intended use is the control of plant diseases they should be considered plant protection products and satisfy the same registration procedures as chemical pesticides. A specific problem arises for plant extracts which can be composed of an association of many different molecules. This is the case for the neem extracts in which 50 different molecules may be present. Azadirachtine, which is one of the major constituents, has already been studied and can be commercialized on its own merit. But organic farmers tend to prefer the natural plant extracts, the composition of which varies depending on the origin of the plant and on the extraction process.

Cultural practices contributing to biological control

Prophylactic methods

Prophylactic methods include those that are useful for preventing the introduction of a pathogen and limiting its epidemic development in a given area. These methods, which are compulsory for quarantine, are also useful to control widely distributed pathogens. It is essential to start a crop from healthy seeds, or healthy transplants. An interesting method to keep seedlings grown in peat or rockwool plugs free of pathogenic organisms is 'biotization' which consists of the introduction of beneficial microorganisms into the growing substrate before sowing the plant. This is particularly important for greenhouse crops, often grown in soilless substrates free from pathogens at the beginning of the growing period. More and more frequently, greenhouses are equipped with insect-proof nets which prevent the entrance of insects responsible for the spread of viral diseases, and with apparatus to disinfest the recirculating nutrient solution. It is advisable to eliminate diseased plants to prevent the build-up of pathogen populations, and to clean equipment before entering a new field. These methods can be qualified as 'good sense' methods, but they are too often neglected.

Crop rotation and residue management

A long-term crop rotation will prevent the build-up of inoculum, especially of the primary inoculum which is important in the case of monocyclic diseases such as most soil-borne diseases. There are a few exceptions to this general law that monocropping will increase disease. The best known example is that of take-all decline: after increasing during a few years (4–5) disease severity will decrease, to such a level that the yield will not be affected by the disease (Hornby, 1998). Most of the diseases induced by soil-borne plant pathogens can be controlled by an appropriate crop rotation sequence, but today economical constrains force farmers to repeatedly grow the same plant species on the same land. An experiment recently performed in our laboratory (Guillemaut, 2003) illustrates the role of the previous crop and of residue management in the case of sugar beet rot induced by *Rhizoctonia solani* AG2-2. Sugar beets were cultivated in a soil infested by a pathogenic strain of *R. solani* AG2-2 after soybean or maize cultivation. Crop residues from the previous crop were either removed or left on the soil surface. Where crop residues had been eliminated, disease incidence on sugar beet was greatly reduced after soybean, but were higher after maize. But the main effect observed was that of the crop residues. The presence of the maize crop residues dramatically increased disease incidence, but not the soybean crop residues. An hypothesis that needs to be confirmed, is that maize is a 'healthy carrier' of *R. solani*, which colonizes the plant without inducing symptoms. The resting structures of *R. solani* find a suitable niche in the crop residues to survive during winter and favourable nutrients to grow saprophytically until the next season. Soil tillage practices also affect the incidence of Rhizoctonia root rot, take-all and the cereal cyst nematode in wheat and barley in Australia (Roget et al., 1996). The role of residue management and other cultural practices in relation to disease incidence has been recently reviewed (Alabouvette et al., 2004); it is difficult to make any generalization from known examples, but it is obvious that much more attention should be given to soil tillage and residue management in relation to disease control.

Solarisation

Solarisation or solar heating is a method that uses the sun's energy to increase soil temperature and so reach levels at which many plant pathogens will be killed or sufficiently weakened, in order to obtain significant control of diseases. Solarisation does not destroy all soil microorganisms, but modifies the microbial balance in favour of beneficial microorganisms. Indeed, many papers report situations where the efficacy of soil solarisation is not only due to a decrease in the pathogenic populations but also to an increase in the density and activity of populations of microorganisms antagonistic to pathogens. Several review papers describe both the technology of solar heating and the mechanisms involved in the control of pests, pathogens and weeds by soil solarisation (DeVay et al., 1991; DeVay, 1995; Katan, 1996). Soil solarisation has a large spectrum of activity; it controls fungi, nematodes, bacteria, weeds, arthropod pests and some unidentified agents, and often results in increased yields when applied to monoculture soils where specific pathogens have not been identified. In this case, solarisation probably controls weak pathogens or deleterious microorganisms responsible for reducing soil productivity, a phenomenon sometimes described as 'soil sickness' (Bouhot, 1997).

Another interesting property of solarisation is its long-term effect. Disease control and yield increase have been reported two and sometimes three years after solarisation. This long-term effect is probably due to both the reduction of the inoculum density and some induced level of suppressiveness of the soil (Lopez-Escudero and Blanco-Lopez, 2001). Obviously, not all pathogens have the same susceptibility to solar heating. Although most of the fungi are well controlled, some failures have been reported. Solarisation is effective in warm and sunny areas in the world and particularly under the Mediterranean climate. However, some interesting data have been reported from other regions of the world where solarisation can be applied under plastic frames or in greenhouses.

Biofumigation or biodisinfection

Better adapted to cooler regions of the world, biological soil disinfection is based on plastic tarping of the soil after incorporation of fresh organic matter (Blok et al., 2000). The mechanisms involved in this newly developed technique are not totally understood. Fermentation of organic matter in soil under plastic results in the production of toxic metabolites and anaerobic conditions which both contribute to the inactivation or destruction of pathogenic fungi. Based on the dominant type of mechanisms involved, Lamers et al. (2004) proposed the distinction between (i) biofumigation that corresponds to the use of specific plant species containing identified toxic molecules, and (ii) biodisinfection which refers to the use of high quantities of organic matter resulting in anaerobic conditions mainly responsible for the destruction of the pathogens. Many species of the *Brassicaceae* (*Cruciferae*) family contain glucosinolates, a class of organic molecules that can be hydrolyzed by a group of similar-acting enzymes (myrosinases) in toxic compounds such as isothiocyanates. These compounds, analogous to some chemical fumigants act as biocides in controlling various soilborne plant pathogens (Kirkegaard et al., 1998; Lawrence and Matthiessen, 2004). Traditionally, to avoid problems when brassicas are used as feed for livestock, plant breeders have selected varieties with reduced levels of glucosinolates. On the contrary, cultivars of *Brassicaceae* with a high content in glucosinolates have now been created. Some of them are already available on the market, specifically for biofumigation.

Plants belonging to the *Alliacae* family also contain molecules with either a direct or an indirect effect on pests and pathogens. Degradation of garlic, onion, and leek tissues releases sulphur volatiles such as thiosulfinates and zwiebelanes which are converted into disulfides having biocidal activities against fungi, nematodes and arthropods (Arnault et al., 2004). In addition to the effects of these toxic compounds, incorporation of high rates of organic matter in soil followed by plastic tarping result in anaerobic conditions that are also deleterious to many pests and pathogens which need aerobic conditions to survive. Block et al. (2000) reported a drastic reduction in the population density of *F. oxysporum* f.sp. *asparagi* and *R. solani* after addition to soil of either ryegrass or cabbage. These promising methods need to be implemented under various situations to define their conditions of use, both their benefits and their limits. But their use will probably require some changes in the cropping sequence, since the land will not be available for cropping for several weeks during the year. However, in our opinion, these methods will gain popularity since disinfection with methyl bromide has now been banned.

Compost amendments

At the present time, European regulations require the recycling of wastes. For organic wastes, composting is an interesting process since it transforms organic waste which can then be used in agriculture. This is a biological process characterized by a heat peak which destroys thermo-susceptible microorganisms, resulting in compost free from most plant pathogens (Bollen, 1985a, b; Coventry et al., 2004). These composts contain some nutrients, especially micro-elements, which improve soil fertility, and most of them possess some capacity to increase soil suppressiveness to diseases induced by nematodes (Lumsden et al., 1983; Oyarzum et al., 1998; Erhart et al., 1999; Widmer et al., 2002) as well as disease suppression in horticultural crops (Cotxarrera et al., 2002; Hoitink and Boehm, 1999; Tilston et al., 2002). For example, Hoitink (1980) developed a growth medium based on composted bark to grow rhododendron and azaleas. This substrate is suppressive towards root rots induced by several species of *Pythium* and

Phytophthora. However, there is no universal rule; the level of disease control obtained depends on many factors such as the chemical properties of the parent materials, the composting process, the types of microorganisms that colonized the compost after the heat-peak and obviously the type of plant pathogens to be controlled. This is probably why published results vary considerably regarding the efficacy of disease control obtained by compost amendment of soil. Based on the results obtained under the frame of two European supported projects (Recoveg and Compost Management in Horticulture) it can be concluded that composts from different origins controlled some diseases but not others. The only exception is Fusarium wilt which is controlled by almost all composts. Compost addition to a soil always results in a stimulation of microbial activities, reinforcing the phenomenon of 'general suppression' towards pathogens (Cook and Baker, 1983). Therefore *F. oxysporum,* which is very susceptible to competition for nutrients (Alabouvette, 1986), is controlled by soil amendment with composts (Serra-Wittling et al., 1996). Other pathogenic fungi, such as *R. solani,* which is able to efficiently utilize the organic matter provided by composts might be favoured by its addition to soil (Steinberg et al., 2004). Many more studies should be devoted to compost and soil microbial ecology to fully understand the mechanisms involved and to enable the correlation of changes in microbial activities with changes in the level of soil suppressiveness to diseases (Pérez-Piqueres et al., 2005).

To enhance the suppressive potential of composts and thus improve the efficacy of disease control, it has been proposed to enrich these composts with specific strains of BCAs. Although promising, this strategy has not yet been successfully applied. Composts possess a certain level of suppressiveness towards introduced microorganisms, so it is quite impossible to establish BCAs in mature composts. The only solution would be to introduce the antagonists after peak heating when there is a certain microbiological vacuum in the composts. Although often proposed, this method is still not adopted by compost producers, since it is difficult to standardize. One of the main constraints is the need to produce composts with constant properties. It is therefore necessary to develop methods of quality control to permit assessment of not only the physico-chemical properties but also the microbiological quality of the composts.

Conclusion

Present status of biological control

Despite progress made in the understanding of the modes of action of BCAs, biological control of plant diseases in Europe is still very limited. As stated above there are only a few products registered in Europe in comparison to hundreds of preparations registered in other countries, especially in the USA. One reason for this difference in usage of biological control is the difference in regulation. In Europe, the placing of plant protection products on the market is regulated by Council Directive 91/414/EEC. Plant protection products consisting of microorganisms as active substances are submitted to that Directive which has been modified by Commission Directive 2001/36/EC to specify requirements for microorganisms. First, the active substance, i.e. the strain of microorganism, has to be clearly identified and its biology described. Then it has to satisfy toxicity tests to characterize its potential effects on human health and its effects on non-target organisms. These aspects are common to the requirements in other countries such as North America and Australia, and the test guidelines are mostly those recommended by OECD or USEPA. But in Europe, the second aspect of the dossier requires demonstration of the efficacy of the preparation, i.e. the formulated product. In European countries, the authorization for putting a plant protection product on the market is given only for specific use for specific crops. Thus, a preparation should be evaluated in several experiments, in different geographic regions, for two consecutive years. This is the main difference between the European regulations and those of other countries. Too often biological control products fail to show a consistent effect under variable environmental conditions. At the present time several active substances which have satisfied the toxicity requirements are still not registered because they have not shown consistent efficacy. This regulation also prevents a product registered for a given use to be used for another purpose on other crops. For example 'Serenade', a preparation based on a strain of *Bacillus subtilis*, which is used in the USA against several diseases in many crops, will be registered in Europe only for specific use.

One could question why products which have not been able to satisfy the requirement of consistent efficacy are used by farmers in countries outside Europe. Obviously there is a difference of approach in countries such as the USA, where the market is supposed to separate good from bad products, and European countries which are less liberal in this domain. But scientifically, it must be admitted that the lack of consistency of biological products is a true problem that must be addressed. Many more studies should be devoted to the conditions of application required for successful biological control of diseases.

The category of 'growth strengtheners' which previously existed in some European countries does not exist anymore at the European level. It means that some *Trichoderma*-based products, largely used in the USA, cannot be registered for this type of use in Europe. It must be underlined that the strain of *Trichoderma* T22 has been extensively studied for its antagonistic properties against several plant pathogens, but almost nothing is known about its mode of action as a growth strengthener (Harman et al., 2004a). This discrepancy between knowledge about the antagonistic activity of this strain and its use for a different purpose is also raising the following questions for scientists: Does growth promotion result from the antagonist activity of this strain? If so, why have the mechanisms of growth promotion not been studied *per se*, and are there unknown modes of action for this strain?

The European situation might not be so different from that in other countries if we consider the 'black market'. Indeed, many non-registered biological control products are used by farmers. These products are those available in other countries, such as plant extracts or BCAs produced by small companies who are not willing or able to fund the studies required for registration. For example, an extract of *Renoutia saccalensis* gave consistent control of powdery mildew on tomato (Trottin-Caudal et al., 2003), but to our knowledge, the company is not willing to register the product. Many different strains of *Trichoderma* are available, some of them are reported to have good efficacy but without registration, there is no warranty, and in most cases there is even no experimental data available. In such a situation, how can farmers make a choice between different products based on *Trichoderma*? It is our opinion that the European regulations might be too strict, but that evidence of efficacy should be provided before putting a biological control product on the market.

Future of non-chemical control methods

Today, consumers want to buy healthy food, free from pesticide residues, but growers do not have the required technologies to control pests and diseases without the use of chemicals. How can we solve this problem? We have to look back at the history of research on biological control and try to find new approaches for its application.

In the field of soil-borne plant pathogens, it is interesting to read again the papers presented during the first symposium held in Berkeley in 1965. All the basic concepts were already developed. There were two approaches to biological control: enhancement of natural populations of enemies or antagonists and introduction of selected strains of BCAs. It appears that the first approach has been neglected and most research efforts have been devoted to the selection of effective antagonists and to the development of plant protection products based on a single strain of a BCA. Putting a product on the market appeared the best way to promote biological control. In our opinion it was a mistake; BCAs are living organisms and cannot be handled and applied as chemical pesticides. Progress made in biochemical and molecular technologies have made possible the elucidation of the modes of action of BCAs at the cellular and molecular levels. Based on this new knowledge it is now possible to create improved strains of BCAs, expressing several modes of action or to transfer into plants some genes involved in biocontrol activity to increase the resistance of the plant. Although promising, not one of these new tools, with the exception of *Bt* maize, is being used in practice. To utilize BCAs successfully it will be necessary to put more effort into applied research aimed at understanding the conditions required for efficacy under changing environmental conditions. Until now the fitness of these organisms has rarely been studied. The formulation needs also to be based on physiological studies of the BCAs to find a compromise between long shelf life and easy handling and practical efficacy. Finally, application of BCAs alone will probably never solve the problem. It is absolutely necessary to integrate microbiological control with other control practices.

Today, there is a renewed interest in alternative strategies which consist in enhancing the natural biocontrol mechanisms which exist in the environment. We certainly have to learn from organic farming practices, how to obtain a natural equilibrium that favours both antagonistic populations and stimulates natural plant defence reactions. Obviously this strategy is much more difficult to follow than a systematic application of pesticides or BCAs. It will not provide ready-to-use tools applicable in any situation. On the contrary, it involves ecological studies to determine the most suitable practices in a given geographical region. It also implies that the cropping system at the farm level and probably at the regional level must be taken into consideration, rather than with individual crops separately. Thus much more research should be directed towards understanding the interactions among plant, pathogens, antagonists, the environmental conditions and farm practices in a given ecological region. The aim of such an integrated approach should be the development of models needed to provide decision-making tools at the farm level. To illustrate this approach, we are currently trying to describe with as many parameters as possible the changes affecting soil microbial communities in response to some practices such as biofumigation or compost amendments, with the aim of identifying factors which can be used as being representative of 'soil quality' (Pérez-Piqueres et al., 2005). Thus we should be able, following the development of these parameters to advise farmers concerning the best practices to maintain or even to improve the quality of the soil.

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