Management of Diseases Induced by Soil-Borne Pathogens, Solarization and Biological Control

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إدارة الأمراض التي تسببها جراثيم التربة والتشميس والمكافحة الحيوية

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الخلاصة: تعتبر الأمراض التي تسببها الجراثيم التي تعيش ضمن الأمراض التي تصعب مكافحتها ولا مناص من إتباع الوسائل التي تمنع دخول هذه الجراثيم إلى الترب غير المصابة حيث أن إزالتها من الترب الملوثة قد تكون مستحيلة، خاصة وأن تطهير التربة باستخدام المواد القاتلة مثل بروميد الميثيل قد فشلت في تحقيق ذلك الهدف، هذا بالإضافة إلى أن هذه المواد ضارة بالإنسان والبيئة. ليس هناك من بد في أن يفضل المزارعون استخدام تقنيات حديثة لا تضر بالبيئة مثل تطهير التربة بواسطة عمليتي التشميس والمكافحة الحيوية حيث تؤدي هاتان الطريقتان إلى إحداث تغيرات في التوازن الميكروبي وتقلل من كثافة الجراثيم وتشجع تنشيط بعض الأحياء الدقيقة المفيدة. لقد ساعدت الدراسات التي أجريت على الترب التي تقل فيها الأمراض التي تسببها الجراثيم التي تعيش في التربة في تعميق الفهم الصحيح للتفاعل بين الأحياء الدقيقة المسببة للأمراض وتلك التي تفتك بها وقد أدى ذلك إلى إمكانية إختبار العناصر التي يمكن إستخدامها في عمليات المكافحة الحيوية.

ABSTRACT: Diseases induced by soil-borne plant pathogens are among the most difficult to control. Prophylactic methods aiming at preventing the introduction of pathogens in healthy soils have to be respected, because it is almost impossible to eradicate pathogens from an infested field soil. Even the drastic disinfestation techniques based on the application of biocide molecules such as methylbromide failed to eliminate the pathogens, but are harmful to man and the environment. Growers should prefer new, environment friendly techniques such as solarization and biological disinfestation of soils. These methods induce changes in the microbial balance, reducing the population density of the pathogens and stimulating the activity of some beneficial microorganisms. The study of soils that naturally suppress diseases induced by soil-borne pathogens has led to a better understanding of the interactions between pathogenic and antagonistic microorganisms and has resulted in the selection of biocontrol agents.

Diseases induced by soil-borne plant pathogens cause extensive damage to many types of crops, all over the world. They result in significant losses affecting both yield and quality. Several types of organisms, bacteria, fungi, actinomycetes and nematodes, living or surviving in the soil are able to attack the root and colonize the plant to some extend. The development of these pathogens may be limited to the underground parts of the plant where they determined local symptoms of root-rot, root-necrosis, crown-rot, galls and nods. Some of these pathogens are able to get into the vessels of the xylem and invade all the vascular system inducing generalized symptoms of yellowing and wilting. Diagnosis of soil-borne diseases is often difficult because symptoms on aerial parts of the plant are usually not characteristic of the pathogen responsible for the disease. Moreover, besides the pathogens inducing well described diseases many other organisms designed as minor or weak pathogens are able to weaken the development of the plant that appears stunted, without obvious symptoms. These deleterious organisms are probably responsible for greater yield reduction than usually suspected.

The frequency and severity of soil-borne diseases have been favoured by cultural practices used in intensive cropping systems. The lack of crop rotation, reduced tillage, reduced organic amendment, the use of new varieties with a very high yield potential, intensive irrigation, frequent applications of pesticides are common practices that have induced changes in the microbial balance, favouring the build-up of pathogen populations and reducing the activity of some beneficial microorganisms.

To control soil-borne diseases, the use of resistant varieties is obviously the best method. But when these resistant varieties do not exist or are not available, intensification of the chemical control has been, too often, the only solution chosen by farmers. In many cases, chemical applications are not sufficient to control satisfactorily the soil-borne pathogens and have detrimental effects on the soil fertility and the environment. During the ten last years there was a trend to adopt new control strategies, based on the integration of several practices including application of reduced dosage of pesticides, soil solarization and biological control.

Many good review papers are already available that discuss in detail the recent progress made in soil solarization and biological control of soil-borne plant pathogens (DeVay, 1995; Katan, 1996 ; Whipps, 1997; Schippers, 1992 ; Lumsden and Vaughn, 1993; Deacon and Berry, 1993).

This article only aims at summarizing the principles on which are based control strategies, inviting those who are interested to read the original papers.

Prophylactic Methods

Before presenting some new methods of control of soil-borne plant pathogens, it is absolutely necessary to recall the traditional methods, especially the prophylactic ones, that are too often neglected, although very useful, when correctly applied. It must be stressed that after introduction into the soil, a soil-borne pathogen will survive for many years. Even if its population density decreases below the detection threshold, the pathogen is still present and will grow again as soon as a host-plant is cultivated. Therefore, it is important to prevent the introduction of the pathogen into an healthy field. It is recommended to use healthy seeds or planting material to prevent the extension of the disease to areas where it has not been detected before. A good example concerns the Bayoud, the fusarium wilt of date-palm. This disease appeared at the end of last century in Morocco and is extending slowly to Eastern North Africa. It is very important to preserve the healthiness of oases of Tunisia and Eastern Algeria by forbidding the transport of palm material from an infected area and by producing new planting material under well controlled conditions (Fernandez *et al.* 1997).

Even when the soil is already infested by a given pathogen it is important to use healthy or treated seeds because introduction of the pathogen with the seeds will surely result in an early and severe disease.

As stated above, when they are available, genetically resistant or tolerant varieties have to be preferred to susceptible ones. When such varieties do not exist, it may be possible to graft the commercial susceptible variety on a resistant rootstock. This well known technique is not only recommended for trees but also for some annual crops such as tomato, eggplant or melon (Laterrot *et al.* 1988). Special varieties have been developed to be used as rootstock for tomato and eggplant, they confer the resistance to several soil-borne pathogens, including fungi, bacteria, nematodes and viruses. The manpower cost of grafting may seem too expensive, but it has to be compared to the advantages; grafted plants not only resist diseases but usually give higher yield for a longer period than nongrafted plants.

Many soil-borne diseases would be economically controlled by a crop rotation. Indeed, as stated above, the crop rotation will not eliminate the pathogen from the soil, but the population density will decrease to a level inducing a disease intensity below the economical threshold. It is well established that the take-all decline of wheat due to *Gaeumannomyces graminis* var *tritici* is usually controlled by a sequence of crop including a non-host plant every two years (Cook and Veseth, 1991).

Many other cultural practices may contribute to decrease the intensity of soil-borne diseases. It is generally more interesting to keep on with the disease rather than to try to eradicate the pathogen through drastic methods.

Soil Solarization Versus Chemical Disinfestation

PHYSICAL AND CHEMICAL DISINFESTATION: Thirty years ago, in intensive vegetable cultures under greenhouses, heat-treatment by soil steaming was a common practice. Most of the pathogens are highly susceptible to heat, the lethal temperature for pathogenic fungi corresponding to 55 to 65°C for 15 to 30 minutes (Bollen, 1969). With the oil crisis, the cost of soil steaming became too expensive and the growers moved to methyl-bromide applications. Methyl-bromide is also a biocide killing every types of organisms and not only the pathogens. Its frequent application results in harmful modifications of the microbial balance. Many beneficial microorganisms such as nitrogen fixing bacteria, mycorrhizal fungi, antagonistic bacteria and fungi are destroyed. The empty space created by methyl-bromide application favours a rapid colonization both from the top and the bottom of the disinfested soil layer. The accidental reinfestation by a pathogen will result in a dramatic severity of the disease since the saprophytic microflora having some antagonistic effects has been destroyed by the fumigation. Moreover, as well established now, methyl-bromide has many deleterious side effects: it is hazardous for the applicators, the growers and is partly responsible for the destruction of the ozone layer.

For all these reasons, methyl-bromide applications will be banned in the near future. It is urgent to propose alternative methods to control soil-borne diseases since several important crops are still totally dependent on methyl-bromide soil fumigation. It is the case for strawberries, trees nurseries and vegetables under greenhouses in many countries. Other chemicals, mainly nematicides applied to soil, also have deleterious side effects on non-target organisms and usually induce changes in the microbial balance of the soil resulting in a decrease of soil fertility. Fortunately, based on the results acquired by the public research organisms, alternative methods of control are being implemented in commercial fields and some of them such as solarization have already been adopted by growers at least in some countries.

SOLARIZATION: Solarization or solar heating is a method that uses the solar energy to enhance the soil temperature and reach levels at which many plant pathogens will be killed or sufficiently weakened to obtain significant control of the diseases. This method is very interesting because, in contrast to chemical disinfestation of the soil, it does not destroy all the soil microorganisms, but modify the microbial balance in favour of the beneficial microorganisms. Indeed, many papers report situations where the efficacy of soil solarization is not only due to a decrease of the pathogenic population but also to an increase of the density and activity of some antagonistic populations of microorganisms such as *Bacillus* spp., *Pseudomonas* sp. and *Thalaromyces flavus*. Several excellent review papers are available that describe both the technology of solar heating and the mechanisms involved in the control of pests, pathogens and weeds by soil-solarization (Katan, 1996 ; Katan and DeVay, 1991; DeVay, 1995; DeVay *et al.* 1991). Therefore, only the main interesting points will be summarized below.

Solarization consists in plastic mulching of soil during the summer months in order to reach temperature lethal to most plant pathogens. However, soil solarization is a hydrothermal process; its effectiveness is not only related to the temperature but also to the soil moisture. Indeed, temperature maxima are obtained when the soil water content is about 70 p.100 of the field capacity in the upper layers and the soil should be moist to a depth of 60 cm. Various kinds of plastic films have been used. Polyethylen tarp, as thin as possible (25 to 50 μm) is recommended because it is transparent to most solar radiations and less transparent than some other plastic to terrestrial radiation.

The duration of solarization is an important factor determining the effectiveness of the treatment. The longer the mulching period, the greater the depth of effective activity, the higher the pathogen-killing rates. Usually four weeks are required to achieve control of the diseases. As stated above, disease control results both from the reduction of inoculum density and from increased activity of some antagonistic microorganisms. Depending on the target pathogen one or the other mechanism is predominant.

An important characteristic of soil solarization is its very large spectrum of activity. This method controls fungi, nematodes, bacteria, weeds, arthropod pests and some unidentified agents. Indeed, solarization often results in increased yield when applied to monoculture soils, where specific pathogens have not been identified. In this case, solarization probably controls the weak pathogens or deleterious microorganisms responsible for "soil sickness". All the pathogens do not present the same susceptibility to solar heating, if most of the fungi are well controlled some failures have been reported.

Another interesting property of solarization is its long term effect. Disease control and yield increase have been reported two and sometimes three years after solarization. This long time effect is probably due both to the reduction of the inoculum density and also to some induced level of suppressiveness of the soil.

Obviously, solarization is effective in warm and sunny areas in the world and particularly under the Mediterranean climate. However some interesting data have been reported from cooler regions of the world where solarization may be applied under plastic frames or in greenhouses. Already adopted by farmers in several countries, this environment friendly technique is still in its infancy and many technological progresses may improve its efficacy and its acceptance by growers.

BIOLOGICAL SOIL DISINFESTATION: When solarization can not be applied, another alternative to methyl-bromide disinfestation consists in a biological soil disinfestation. This method is based on plastic tarping of the soil after incorporation of fresh organic matter. The mechanisms involved by this newly developed technique are not totally understood. But it is supposed that the anaerobic fermentation of organic matters induces inactivation or destruction of the pathogenic fungi. This technique has been experimented with success to control fusarium wilt of asparagus (Blok *et al.* 1995). This last example underlines that with some efforts of research, alternatives to chemical soil disinfestation could be developed and applied.

Disease-suppressive Soils

In contrast to soil disinfestation that aims at eradicating the pathogen to control the disease, naturally occurring disease-suppressive soils provide good examples of fields where the disease does not appear despite the presence of the pathogen, a susceptible host-plant and climatic conditions favourable to disease expression. Understanding the mechanisms responsible for soil suppressiveness should lead to new disease control strategies taking into account the microbial balance of the soil and its interactions with the abiotic characteristics of the soils and the cultural practices.

Soils suppressive to some of the most important soil-borne plant pathogens have been described, indicating that soil suppressiveness to diseases is not an exceptional phenomenon. The best experimental procedure to verify the hypothesis that a soil may be suppressive to a given disease consists in introducing increasing concentrations of the pathogen into this soil and to rate disease incidence on a susceptible host in comparison to that produced by the same inoculum concentrations in a conducive control soil. All experimental conditions being similar, differences in disease incidence have to be attributed to differences in the soil environment.

Properties of Soil Suppressiveness

Disease suppression may result from a direct effect of the soil on the pathogen or from an indirect effect through the host-plant. Therefore, Cook and Baker (1983) suggested to distinguish between pathogen-suppressive soils and disease-suppressive soils. However, the mechanisms responsible for soil suppressiveness are very complex; it is therefore difficult to clearly attribute disease suppression to only a direct effect on the pathogen. Although variations of disease incidence related to soil types have been recognized for many years it is only during the last thirty years that mechanisms involved in disease suppression have been discovered. Three examples of suppressive soils have been extensively studied; they concern the take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*, fusarium wilts due to different formae speciales of *Fusarium oxysporum* and root rot of tobacco caused by *Thielaviopsis basicola*. These few examples show a great diversity of mechanisms responsible for disease suppression.

However most of the suppressive soils have several properties in common demonstrating that suppressiveness is fundamentally microbial in nature. Disease suppression results from more or less complex microbial interactions between the pathogen and all or a part of the saprophytic microflora. Indeed, the suppressive effect disappears upon destruction of organisms by biocidal treatments and can be restored by mixing a small quantity of suppressive soil into a previously heat-treated soil (Louvet *et al.* 1976; Scher and Baker, 1980 ; Shipton *et al.* 1973).

Properties that account for the microbiological nature of disease suppression do not demonstrate that soil physio-chemical properties play any role in the mechanisms of suppressiveness. On the contrary, early studies on fusarium wilt suppressive soils established correlations between soil type, presence of smectite clays and soil suppressiveness to fusarium wilts in Central America (Stotzky and Martin, 1963). A documented example of the role of abiotic factors is that of Swiss soils suppressive to black root rot of tabacco ; Stutz *et al.* (1989) established that only soils derived from moraine and containing vermiculitic clay minerals are suppressive to *Thielaviopsis basicola*. Many other abiotic factors such as soil texture, water potential, aeration, pH, organic matter content, cation availability (Al, Fe, Mn) are indirectly involved in the mechanisms of disease suppression, but it is difficult to generalize the findings from one soil to another.

Finally, cultural practices, such as monoculture as opposed to crop rotation, may play an important role in the establishment of soil suppressiveness to soilborne plant pathogens. Several examples demonstrate that monoculture of a susceptible host plant is responsible for the build-up of soil suppressiveness (Baker and Chet, 1982), and the well known phenomenon of take-all decline is the best example of "acquired" suppressiveness. Conversely, suppressiveness to fusarium wilts appears stable with time and has been described as constitutive but it may be not always independent from the cropping history of the soils (Sneh *et al.* 1987). In fact, involvement of abiotic factors or cultural practices is difficult to demonstrate and most of the recent progress concerns the role of microbial populations inhibiting the growth and activity of the pathogen in suppressive soils.

Mechanisms and Microorganisms Responsible for Soil Suppressiveness

Mechanisms and microorganisms responsible for soil suppressiveness to fusarium wilts will be described to illustrate the complexity of phenomena involved in disease suppression. Two complementary approaches have been followed to detect and prove the involvement of specific mechanisms and particular microorganisms in the suppression of fusarium wilts in suppressive soils. First, manipulation of the nutrient status of the soil enabled the demonstration of the role of competition for nutrients in the mechanisms of suppression. Both carbon and iron have been proved to be limiting nutrients. Scher and Baker (1982) modified iron availability in the suppressive soil from the Salinas Valley and demonstrated that the level of suppressiveness was either decreased or increased by addition of FeEDTA or FeEDDHA that respectively make iron more or less available for *F. oxysporum* growth. The same results were obtained by Lemanceau *et al.* (1988) who also demonstrated that addition of glucose increased disease incidence in the suppressive soil from Châteaurenard. In that case, competition for carbon was considered to be most important because addition of glucose resulted in greater disease incidence when the amount of available iron was reduced by addition of EDDHA.

Secondly, manipulation of the microbial balance of suppressive soils enabled the demonstration of the role of specific populations of microorganisms antagonistic to the pathogen. Rouxel *et al.* (1979) established that suppressiveness of the soil from Châteaurenard disappeared after elimination of the populations of nonpathogenic *Fusarium* by heat-treatment and reappeared after their reintroduction into the heat-treated soil. Similarly, Scher and Baker (1980) isolated strains of fluorescent *Pseudomonas* spp. from a suppressive soil from the Salinas Valley and demonstrated that introduction of these bacteria into a conducive soil made it suppressive. Other antagonistic microorganisms have been proposed as having a role in the suppression of fusarium wilts (Alabouvette, 1990) but only populations of nonpathogenic *F. oxysporum* and fluorescent *Pseudomonas* spp. have been repeatedly proved to be involved in the mechanisms of suppression of fusarium wilts, in naturally occurring disease-suppressive soils (Alabouvette, 1990 ; Schippers, 1992).

These two experimental approaches have provided good evidence that competition for nutrients on one hand, and populations of fluorescent *Pseudomonas* spp. and nonpathogenic *F. oxysporum* on the other hand are involved in the mechanisms of suppression. However, these approaches do not demonstrate that the specific antagonistic populations associated with suppression are responsible for the competitive effects demonstrated in the suppressive soils. Kloepper *et al.* (1980) demonstrated that competition for iron resulting from the production of siderophores by fluorescent *Pseudomonas* spp. could be a mechanism of disease suppressiveness. The use of mutant strains of *Pseudomonas* spp. which have lost the capacity to chelate iron, inhibit the growth of *Fusarium* spp. in vitro, and induce suppressiveness in conducive soils, led to the conclusion that production of siderophores by fluorescent *Pseudomonas* spp. is responsible, at least in part, for competition for iron that is more intense in suppressive than in conducive soils.

Since the soil microbial biomass in a suppressive soil was more responsive to glucose amendment than the biomass of a conducive soil, competition for carbon was attributed to the activity of the total soil biomass (Alabouvette *et al.* 1985). Subsequently, it was also established that pathogenic and nonpathogenic *F. oxysporum* are competing for carbon in soil (Couteaudier, 1992). Indeed, the most effective strains of nonpathogenic *F. oxysporum* in biocontrol experiments are those which are the most efficient in glucose consumption (Alabouvette and Couteaudier, 1992).

Finally, Lemanceau *et al.* (1993) demonstrated that competition for carbon and competition for iron are not independent from each other. The intensity of the intraspecific competition for carbon between pathogenic and nonpathogenic *F. oxysporum* depends on iron availability controlled by the activity of siderophore producing *Pseudomonas* spp. In vitro, the pathogenic *F. oxysporum* f.sp. *dianthi* was more sensitive to competition for carbon than the nonpathogenic strain Fo47, and its growth yield (defined as the germ-tube length per unit of glucose consumed) was reduced when the availability of iron was decreased by addition of the pseudobactin produced by a strain of *Pseudomonas putida*. These results provide a good explanation for the synergistic effect of the coinoculation of nonpathogenic *F. oxysporum* with fluorescent *Pseudomonas* spp. to control fusarium-wilt incidence of several crops (Lemanceau and Alabouvette, 1991b ; Lemanceau *et al.* 1992).

Interaction of competition for carbon and iron involving populations of fluorescent *Pseudomonas* spp. and nonpathogenic *F. oxysporum*, show the great complexity of mechanisms responsible for natural suppressiveness of soils to fusarium wilts. Moreover, other mechanisms involving the host-plant, such as systemic induced resistance, also play a role as suggested by Van Peer *et al.* (1991) and Mandeel and Baker (1991). It is evident that stable suppressiveness such as suppressiveness of the soil from the Salinas Valley or Châteaurenard is based on a cooperation among several microorganisms and mechanisms (Schippers, 1992). Although our understanding of the mechanisms of soil suppressiveness is still incomplete, present knowledge allows applications towards biological control of soilborne plant pathogens.

Enhancement of the Natural Level of Suppressiveness to Control Soil-borne Diseases

Two types of strategies can be used to utilize suppressive soils to control soilborne plant pathogens. One strategy is based on enhancement of the level of suppressiveness that exists in any soil. The other strategy consists of the selection of efficient strains of antagonistic microorganisms isolated from suppressive soils and their utilization as biocontrol agents, and will be presented below.

Every soil has a potential for disease suppression. Soil receptiveness to disease may be considered as a continuum from strongly suppressive to highly conducive (Alabouvette *et al.* 1982 ; Linderman *et al.* 1983).

Mechanisms of competition for nutrients, populations of nonpathogenic *Fusarium oxysporum* and populations of fluorescent *Pseudomonas* spp. exist in every soil. However depending on the level and activity of the populations of antagonists and on the intensity of competition, the same types of interactions will result in either conduciveness or suppressiveness of the soil to fusarium wilts. Biocontrol could be achieved by managing the soil environment in order to enhance populations and mechanisms responsible for suppression.

Most attempts to control fusarium wilts by manipulations of the abiotic environment have failed (Alabouvette, 1989). From a theoretical point of view, iron availability is related to pH, therefore application of lime should increase the level of suppressiveness of soils to fusarium wilts. Indeed, most of the suppressive soils have a pH value greater than 7 and, in Florida, liming soils effectively reduces severity of wilts when associated with ammonium nitrogen (Jones and Woltz, 1981).

A correlation has been established between suppressiveness and presence of smectite type clays in suppressive soils. Indeed, introduction of 25% of montmorillonite in a conducive sandy soil made it suppressive to fusarium wilt of flax (Amir and Alabouvette, 1993). Hoeper and Alabouvette (1996) demonstrated that in another conducive soil, illite was more effective that montmorillonite in inducing suppressiveness. This effect was also correlated with increased pH in soil-clay mixture. Clearly, it is not possible to add clays on a practical scale to induce suppressiveness in conducive soils.

A more realistic approach would be the choice of cultural practices favoring the enhancement of the suppressive potential of the soil. Jones *et al.* (1989) reviewed the roles of different macro and micro-elements in relation to fusarium wilts of vegetables and ornamentals. Fertilization sometimes has a clear effect on the reduction of disease incidence, but this generalization is not always true. Most studies involving nutrients did not allow the distinction between the direct effect of the elements on the plant physiology and an indirect effect on the level of soil suppressiveness to fusarium wilts.

The only well documented example of the influence of cultural practices on disease incidence and soil suppressiveness is that of take-all decline due to the monoculture of wheat (Cook and Veseth, 1991). The crop itself, as well as weeds or cover crops, affects the suppressive level of the soil. Field observations in oil-palm groves suggested that soil covered with *Calopogonium* could be more conducive to fusarium wilts than the soil planted with *Puraria* (Abadie *et al.* 1996).

Organic amendments have been proposed to control soilborne diseases (Lumsden *et al.* 1983) although the effect of organic amendments have not been studied in relation to induction of suppressiveness in soil. One exception is the Ashburner system to control *Phytophthora* root rot of Avocado in Australia based on the incorporation of large amounts of organic matter to reproduce the environment of naturally suppressive soils that exist in the rain forest (Cook, 1982).

Clearly, more knowledge is needed before biological procedures based on modifications of soil factors could be proposed confidently.

Biological Control

Having demonstrated the involvement of antagonistic microorganisms in the suppressive effect of some soils to diseases, it was logical to try to utilize the microorganisms to control soil-borne plant pathogens in conducive soils. A review of the literature shows that many types of organisms have been proposed to control soil-borne pathogens and that several mechanisms are responsible for their antagonistic properties.

Modes of Action of Biological Agents

Antagonistic effects responsible for disease suppression results from a direct antagonism directed against the pathogen mainly during its saprophytic phase, or from an indirect action through induced resistance of the host-plant.

Microbial antagonism implies direct interactions between two microorganisms that share the same ecological niche. There main types of direct interactions may be characterized: parasitism, competition for nutrients and antibiosis. These interactions are not exclusive from each other, on the contrary a given strain of biocontrol agent may possess several modes of actions and it is often difficult to distinguish the relative importance of each of them in the efficiency of the observed antagonism. Microbial antagonism mainly occurs during the saprophytic phase of plant pathogens and contributes to reduce the inoculum density and the saprophytic growth of the pathogen in the soil and at the root surface resulting in a decrease of the probability for the pathogen to achieve successful infections of the host-plant.

Parasitism

Parasitism of plant pathogen by other microorganisms including viruses is a well distributed phenomenon, but its significance in relation to biological control of plant diseases is still controversial.

The parasitic activity of strains of *Trichoderma* spp. towards pathogens such as *Rhizoctonia solani* has been extensively studied (Chet and Baker, 1981) and several other mycoparasites such as *Coniothyrium minitens* or *Sporidesmium sclerotivorum* have been tested as biocontrol agents and some of them are efficient in controlling diseases caused by *Sclerotinia* spp. and other sclerotia forming fungi (Adams and

Fravel, 1993 ; Whipps and Lewis, 1980). Since, cell wall degrading enzymes such as chitinases and glucanases are involved in the process of parasitism of several pathogenic fungi by *Trichoderma* and because strains of *Trichoderma* also produce several types of toxic molecules, the boundary between parasitism and antibiosis is not clearly established and more than a single mode of action is responsible for the biocontrol activity of *Trichoderma* spp.

Competition for Nutrients

As already stated competition for carbon is one of the mechanisms responsible for soil suppressiveness to fusarium wilts, but competition for carbon is expressed in every soil and is considered as responsible for the well-known phenomenon of fungistasis (Lockwood, 1977) describing the inhibition of fungal spore germination in soil. Energy deprivation in soil is also partly responsible for « general suppression of a pathogen which is directly related to the total amount of microbiological activity at a time critical to the pathogen » (Cook and Baker, 1983). One must remember that any biocontrol agent applied to soil will be submitted to soil fungistasis and may be unable to express its antagonistic activity. But, some species or strains of antagonists are more competitive than other, and should be selected for biological control. For example, Couteaudier and Alabouvette (1990) have shown that a great diversity exists among strains of nonpathogenic *F. oxysporum* in relation to their ability to utilize carbon efficiency. A significant correlation was established between the ability of several strains of nonpathogenic *F. oxysporum* to inhibit the germination of the pathogen in the rhizosphere, reduce disease incidence of fusarium wilt of flax and their ability to complete efficiently for carbon, with the pathogenic *F.o.* f.sp. *lini* in the soil (Couteaudier, 1992).

Competition for carbon has also been involved in the determinism of the antagonism expressed by different strains of *Trichoderma* sp. against several plant pathogens, especially *F. oxysporum* (Chet, 1990).

Competition for minor elements also frequently occurs in soil. As already stated (see above) 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. Under conditions of iron stress, these bacteria synthetise siderophores, called pseudobactins or pyoverdines which show a higher affinity for Fe^{+++} than fungal siderophores. Numerous studies have associated the bacterial antagonism to pseudobactin synthesis and several review papers are available (Leong, 1986; Schippers *et al.* 1987; Bakker *et al*, 1991; Lemanceau and Alabouvette, 1993).

Competition for other nutrients may also occur and competition for Mn, Zn or Cu has been proposed as a mechanism contributing to the control of certain diseases. Competition for a given nutrient is not exclusive from competition for another nutrient and therefore association of two antagonistic microorganisms competing with the pathogen for two different nutrients may result in an increased efficacy of biocontrol, as demonstrated by the association of a strain of nonpathogenic F.oxysporum competing for carbon with a strain of fluorescent pseudomonad competing for iron, with the pathogen in the rhizosphere of the host-plant.

Antibiosis

Antibiosis is the antagonism resulting from the production by one microorganism of secondary metabolites toxic for another microorganism. Antibiosis is a very common phenomenon responsible for the biocontrol activity of many organisms such as *Pseudomonas* spp., *Bacillus* spp. or *Trichoderma* spp. developed as biocontrol agents.

A variety of different antibiotics, bacteriocins, enzymes, and volatile compounds have been described and are involved in the suppression of different pathogens. Several review articles are available (Fravel, 1988 ; Loper and Lindow, 1993 ; Weller and Thomashow, 1993; Alabouvette *et al.* 1996a). A given strain of biocontrol agent may produce several types of antifungal compounds, effective against certain species of fungal pathogens. For example, the production by fluorescent *Pseudomonas* sp. of phenazines and 2-4-diacetylphloroglucinol was shown to be the primary mode of antagonism against *Gaeumannomyces graminis* var. *tritici* (Vincent *et al.* 1991 ; Keel *et al.* 1992 ; Harrison *et al.* 1991), but 2-4 diacetylphloroglucinol and cyanide were involved in the antagonism expressed against *Chalara elegans* (Keel *et al.* 1990 ; Keel *et al.* 1992 ; Voisard *et al.* 1989). On the contrary these secondary metabolites have not been yet implicated in the inhibition of the growth or activity of *F.oxysporum* (Lemanceau and Alabouvette, 1993).

Since a strain often produces several of these metabolites the best procedure to demonstrate the involvement of a given molecule in the antagonistic activity of the biocontrol agent is to produce mutants affected in their ability to synthesize the molecule and to demonstrate that the deficient mutant is no more able to control the disease. But it is important to emphasize that a single antifungal metabolite generally does not account for all the antagonistic activity of a biocontrol agent.

Induced Systemic Resistance

More and more studies are devoted to the resistance induced in the host-plant by application of biocontrol agents. Induced systemic resistance classically occurs when an inducing agent is applied prior to challenge inoculation with a pathogen, resulting in reduced disease in comparison to the non-induced control.

Kuc *et al.* (1987) reported systemic protection of cucumber against *Colletotricum orbiculare* when the cotyledons or the first leaves of the plant were pre-inoculated with the same pathogen. It has also been well established that the pre-inoculation of an host-plant with an incompatible *forma specialis* or race of *F.oxysporum* will result in reduced disease severity when the plant is inoculated with the compatible pathogen (Biles and Martyn, 1989). Therefore it was suggested that the nonpathogenic fusaria used to control fusarium wilts may be effective through induced resistance (Mandeel and Baker, 1991). Using one experimental design with a splitted root system allowing to apply the biocontrol agent on one side and the pathogen on the other side, Olivain *et al.* (1995) demonstrated that induced systemic resistance contribute to the biocontrol efficacy of a nonpathogenic strain of *F. oxysporum*.

The fluorescent pseudomonads selected for their plant growth promoting capacity or for their biocontrol activity have been shown to induce systemic resistance in the plant (Kloepper *et al.* 1996). The first evidence was given by Van Peer *et al.* (1991) who demonstrated that root colonization of carnation by a strain of fluorescent *Pseudomonas* resulted in an accelerated and increased accumulation of phytoalexins in the stem of carnation after inoculation with *F.o.* f.sp. *dianthi*.

Many other biocontrol agents are able to induce resistance in the host-plant and several recent review papers are available (Kloepper *et al.* 1996 ; Kuc, 1987; Van Loon, 1996). Induced resistance is not exclusive from other modes of action and may exert a complementary effect to microbial antagonism.

Application of Biocontrol Agents

SCREENING: The first step in developing a biocontrol method is the screening of an effective strain of biocontrol agent. Two different approaches can be followed to screen microbial strain.

The first one is based on a preselection on specific modes of actions using *in vitro* assays designed to reveal specific metabolites such as siderophores or antibiotic production. The limit of such a strategy is that the selection is only made on known mechanisms and that the metabolites produced *in vitro* are not necessarily synthesised in the rhizosphere. There is usually a poor relation between the ability of an antagonist to suppress the pathogen growth *in vitro* and its ability to suppress the corresponding disease *in vivo*. In any case, the *in vitro* preselection must be followed by an evaluation of the strains to suppress the diseases *in vivo*.

The second approach to select efficient strains consists in evaluating the efficacy of each strain in a bio-assay where the antagonist is confronted with the pathogen in the soil environment in the presence of the host-plant. Such an experimental procedure using flax and its pathogen *F.oxysporum* f.sp. *lini* has been developed to screen for efficient strains of nonpathogenic *Fusarium*. The candidate strains are grown *in vitro* and mixed into a soil. After 2 weeks of incubation, the pathogenic *Fusarium oxysporum* is added at increasing concentrations of inoculum and a susceptible variety of flax is sown. Disease incidence is recorded every week and the efficacy of the strains is compared using a statistical method based on the calculation of the plant survival probability, which is a function of time, inoculum concentrations and effectiveness of the antagonist (Corman *et al.* 1986). The last step of this screening consists in ensuring that the selected strains effective against fusarium wilt of flax are also effective against fusarium wilts or rots of tomato, carnation, and muskmelon against which they will be used. This type of screening is time and space consuming but gave consistent data and allowed us to select the strain Fo47 which is used in biological control experiments. The same type of procedure was followed to screen for effective strains of fluorescent pseudomonads, but the possibility to use a combination of fluorescent pseudomonad with the nonpathogenic F.oxysporum Fo47 was also considered. Four classes were established based on the effect of the bacteria on disease severity on their own, or in combination with Fo47. Most of them did not modify the percentage of wilted plants, but 10.8% of them significantly improved the control achieved by Fo47 (Lemanceau and Alabouvette, 1991a). This procedure enabled us to select the strain of P.fluorescens C7 which has shown, since then to consistently improve the control, achieved by Fo47 of different fusarium wilts and of crown and root rot of tomato (Lemanceau and Alabouvette, 1991a; Lemanceau *et al.* 1992).

MASS PRODUCTION, FORMULATION AND DELIVERY: Having selected an efficient strain of biocontrol agent, it is necessary to produce large amounts of inoculum to set up large scale experiments. Indeed, fermentation and formulation processes represent important steps in the development of a biocontrol product. Not only a high biomass should be produced at the lowest cost, but the properties of this biomass, *ie* its capacity to control the disease must be conserved during the processing and after the storage and the application. Several review papers are available that discuss the main requirements for producing and formulating an active biomass (Lumsden *et al.* 1995 ; Lewis *et al.* 1991 ; Whipps, 1997). For example, in the case of the nonpathogenic strain Fo47, the production is achieved in fermentors. At the end of the fermentation period, the growth medium is removed by filtration. The microchlamydospores produced are mixed with talcum powder, an inert carrier. This talc inoculum is dried at 20°C by forced air and then stored at 4°C. The propagules of *Fusarium* remains viable for at least 18 months when kept at 4°C and for several months when kept at room temperature. Therefore, this talc inoculum can be commercialised without any specific problems. The inoculum of Fo47 can be mixed with soil directly as a powder or after having been suspended in water (Alabouvette *et al.* 1987).

Another technique of formulation has been recently developed and used for several types of biocontrol agents including bacteria and fungi. The propagules are embedded in alginate pellets (Fravel *et al.* 1985 ; Lumsden and Lewis, 1989). This technique enables to produce a material easy to handle and apply to soil.

An alternative to liquid fermentation is the solid state fermentation, where the biocontrol agent is directly produced on a solid material that provides nutrient and a substratum that helps to solve the problem of formulation. Indeed, the substratum containing the biological control agent may be directly applied to soil. Moreover this technique enables to utilize different types of agricultural waste products, that are relatively cheap and can be found on the local market. For example, *Trichoderma* spp. and *Gliocladium* spp. have been produced in brand-sand or brand-peat mixtures.

All these techniques based on direct incorporation of the biocontrol agents to the soil are not adapted to large field applications. They have mainly be used in horticulture and vegetable production under greenhouses. Seed application seems to be the best approach to make biological control available for open field crops. But coating microorganisms on seeds is not an easy process, since the microorganisms not only must survive but be active at the right time in order to colonize the young radicle emerging from the seed. At least a commercial product « BioCoat » made of a strain of fluorescent *Pseudomonas* sp. has been used to control fusarium wilt of radish, but is no longer available (Alabouvette *et al.* 1996b).

Conclusions

This very short review of methods used to manage soil-borne plant pathogens clearly indicates that many ways other than chemical eradication of the pathogen may be chosen. It is obvious that not a single method will be able to control the soil-borne diseases, it will be necessary to combine several of them to achieve a satisfactory level of control.

For example, introduction of biological control agents may be combined with solar heating of the soil or with biological soil disinfestation. Biological control or solarization may also be combined with application of pesticides at low dosage.

All these methods have also to be combined with other cultural practices aiming at lowering the soil inoculum potential and stimulating the defense reactions of the plant.

The use of molecular technology will also result in the development of new methods based on the application of improved strains of biological agents associating several modes of action or over-expressing beneficial traits.

Creation of new plant varieties less susceptible to pathogens may also help to control diseases. There are already many attempts to introduce into plants microbial genes such as chitinase genes involved in the biocontrol activity of some microorganisms.

It is not possible, in this paper, to review all the literature dealing with these new approaches, but we must be confident that new control strategies will be available in the near future.

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