Modes of Action of Non-pathogenic Strains of *Fusarium oxysporum* in Controlling Fusarium Wilts

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Abstract

Many studies have demonstrated the capacity of non-pathogenic strains of *F. oxysporum* to control Fusarium diseases. These non-pathogenic strains show several modes of action contributing to their biocontrol capacity. They are able to compete for nutrients in the soil, affecting the rate of chlamydospore germination and the saprophytic growth of the pathogen, diminishing the probability for the pathogen to reach the root surface. They are competing with the pathogen at the root surface for colonization of infection sites, and inside the root where they induce plant defence reactions. By triggering the defence reactions, they induce systemic resistance of the plant. Depending on the strain, and on the plant species, these mechanisms are more or less important, leading to a more or less efficient biocontrol efficacy.

Keywords: competition; induced resistance; root colonization

INTRODUCTION

The idea of using non pathogenic strains of *F. oxysporum* to control Fusarium diseases came from the studies of soils naturally suppressive to Fusarium wilts. The existence of soils that naturally limit the incidence of Fusarium wilts has been recognized since the end of the 19th century. But TOUSSOUN (1975) was the first to state that soil suppressive to Fusarium wilts supported large populations of non pathogenic *Fusarium* spp. The same type of observation was made in the suppressive soil from Châteaurenard which harbored high populations of *F. oxysporum* and *F. solani* (LOUVET *et al*. 1976). The involvement of the non pathogenic populations of *Fusarium* spp. in the mechanism of soil suppressiveness was confirmed experimentally by a form of "Koch's postulates" showing that the suppressiveness was destroyed by biocidal treatment eliminating the populations of *Fusarium* spp. and restored by introduction of non pathogenic *Fusarium* spp. in the disinfested soil (ROUXEL *et al*. 1979). Strains of *F. oxysporum* and *F. solani* were much more efficient in establishing suppressiveness in soil than other species of *Fusarium*. Moreover effective biocontrol strains of non pathogenic *F. oxysporum* have been isolated from the stem of healthy plants (OGAWA & KOMADA 1984; POSTMA & RATTINK 1992). Therefore, most of the research dealing with biological control of Fusarium diseases have focused on the modes of action of the non-pathogenic strains of *F. oxysporum*.

Modes of action of non-pathogenic *Fusarium oxysporum*

The mechanisms of action associated with non-pathogenic *F. oxysporum* can be divided in two broad categories: direct antagonism of the non-pathogenic towards the pathogen and indirect antagonism through the host plant. Until now there is no evidence of either parasitism or antibiosis among strains of *F. oxysporum*, but there are very good arguments in favor of competition for nutrients in the soil and at the root surface. Induction of local and systemic resistance within the host plant is another mode of action well documented.

Direct interactions in soil and the rhizosphere

In the absence of any evidence of antibiosis between non-pathogenic and pathogenic strains of *F. oxysporum*, the hypothesis of trophic interactions was proposed to explain the role of non-pathogenic *F. oxysporum* in the

mechanisms of soil suppressiveness. More precisely the hypothesis of competition for the carbon source was proposed based on the fact that a single addition of glucose to a suppressive soil was sufficient to make it conducive (LOUVET *et al*. 1976).

To demonstrate the validity of the hypothesis of competition for carbon, between strains of *F. oxysporum,* COUTEAUDIER and ALABOUVETTE (1990) compared the growth kinetics of a small collection of strains of *F. oxysporum* introduced into a sterilized soil amended with glucose. The modeling of the growth curve (COUTEAUDIER & STEINBERG 1990) enabled to calculate the growth rate and the yield coefficient (i.e., the number of propagules formed per unit of glucose consumed) for each strain. Results showed a great diversity among the 7 strains compared; the yield coefficient varied from 1×10^6 to 8×10^6 propagules formed per mg of glucose consumed. Then 6 of these strains were confronted to the 7th strain, the pathogenic strain *F. o.* f.sp. *lini* (Foln3) resistant to benomyl. Each strain was introduced into the sterilized soil in combination with the pathogenic strain Foln3 at five different inoculum ratios. Following the kinetics of growth of each strain in mixture it was possible to calculate a "competitiveness index" for each strain. These indexes ranged from 1.3 to 3.5, indicating a large diversity in the ability of these 6 strains to compete in soil with the pathogenic strain *F. o.* f.sp. *lini*. LEMANCEAU *et al*. (1993) have confirmed, *in vitro*, that carbon was the major nutrient for which a pathogenic strain of *F. o.* f.sp. *dianthi* was competing in soil-less culture with the biocontrol agent Fo47.

These results were confirmed by LARKIN and FRAVEL (1999) who demonstrated that isolate Fo47 significantly inhibited pathogen chlamydospore germination in soil at glucose concentration of 0.2 mg/g of soil and greater. In addition, germ tube growth also was significantly reduced in soil containing Fo47 compared with untreated soil.

Competition for nutrients has been showed to be involved in the mode of action of other isolates of non-pathogenic *F. oxysporum* such as strain 618.12 (POSTMA & RATTINK 1992) and strains C5 and C14 (MANDEEL & BAKER 1991).

Direct interactions on the root surface

The second place where competition can occur is the root surface. MANDEEL and BAKER (1991) stated that the root surface presented a definitive number of infection sites that could be protected by increasing the inoculum density of the non-pathogenic strain. OLIVAIN and ALABOUVETTE (1997, 1999) clearly showed, using Gus transformed strains, that both a pathogenic and a non-pathogenic strain were able to actively colonize the surface of the tomato root. The main spots of colonization were the root apexes, the elongation zone and the points of emergence of secondary roots, in fact, all the zones were exudation of nutrients is abundant. Both strains colonizing the same spots at the root surface one can assume that the more competitive will colonize a greater surface of the rhizoplan than the less competitive strain. NAGAO *et al*. (1990) showed that different strains of *F. oxysporum* not only have different capacities to colonize a heat-treated soil, but also the surface of flax roots grown in the colonized soil. There was no correlation between the population density of the biocontrol strains in soil and their capacity to effectively colonize the roots, indirectly demonstrating that each strain has its own capacity to colonize the surface of the root. All together these observations support the existence of competition among strains of *F. oxysporum* for the colonization of the surface of the plant root.

Competition in the root tissues and locally induced resistance

OLIVAIN and ALABOUVETTE (1997, 1999) also showed that both the pathogen and a non-pathogenic strain were able to penetrate the epidermal cells and colonize the cortex to some extend. The plant reacted to this fungal invasion by expressing defense reactions (wall thickenings, intracellular plugging etc.) that were more intense in the case of the non-pathogen. As a result these defense reactions always prevented the non-pathogen to reach the stele, although the pathogenic strain grew quickly towards the vessels, which were invaded. When both strains are present in the same root, the more intense defense reactions induced by the non-pathogenic strain can contribute to limit colonization by the pathogen (BENHAMOU & GARAND 2001). These observations are in agreement with previous results obtained by EPARVIER and ALABOUVETTE (1994) who utilized another approach to demonstrate that pathogenic and non-pathogenic strains were competing for root colonization. In these experiments showing a limited colonization of the root by the pathogen in the presence of the non pathogen, it is very difficult to determine the relative importance of direct fungal competition and indirect interaction mediated through local defense reactions of the plant. Similarly, POSTMA and LUTTIKHOLT (1996) considered the hypothesis of a direct competition between

two strains of *F. oxysporum* in the vessels of the host plant. They showed that some non-pathogenic strains were able to reduce the stem colonization by the pathogen *F. o.* f.sp. *dianthi*, resulting in a decrease of disease severity and concluded that either locally induced resistance or direct competition between strains within the vessels could cause this disease suppressive effect.

Systemic induced resistance

It has been established for many years that pre-inoculation of a plant with an incompatible strain of *F. oxysporum* resulted in the mitigation of symptoms when the plant was, later on, inoculated with a compatible strain (MATTA 1989). This phenomenon was described as cross protection or premunition. Today this phenomenon is considered as an expression of induced systemic resistance, a general mechanism of plant response to microbial infection or stresses from various origins. This induced systemic resistance (ISR) is extensively studied since it could explain the disease control provided by non-pathogenic strains of *F. oxysporum*. BILES and MARTYN (1989) were the first authors to attribute to ISR the control of Fusarium wilt of watermelon achieved by several strains of non-pathogenic *F. oxysporum*. Many papers reported experiments where a non-pathogenic strain applied to some roots of a host plant can delay symptom expression induced by the specific pathogen separately applied to other roots or directly into the stem of the plant (BILES & MARTYN 1989; FUCHS *et al*. 1997; KROON *et al*. 1992; LARKIN & FRAVEL 1999; MANDEEL & BAKER 1991; OLIVAIN *et al*. 1995). Since this split-root method prevented any direct interaction between the two microorganisms, the protection has to be attributed to increased plant defense reactions in response to root colonization by the non-pathogenic strain.

Another way to demonstrate the importance of ISR in the mechanism of action of non-pathogenic *F. oxysporum* is to study the dose response relationship in relation to effective control of the disease. LARKIN and FRAVEL (1999) showed that the strain Cs20 was able to control disease induced by a high inoculum density of the pathogen even when it was applied at a low concentration.

There is good evidence that ISR is one of the mechanism by which non-pathogenic *F. oxysporum* control Fusarium disease, but in contrast to other plant microorganism models where ISR has been clearly correlated with an increased production of plant enzymes involved in plant resistance, there is a lack of evidence for these mechanisms being involved in the case of plant inoculated with non-pathogenic strains of *F. oxysporum*. TAMIETTI *et al*. (1993) were the first authors to show an increased activity of several plant enzymes related to plant defense reactions in tomato plants transplanted in sterilized soil infested with a strain of non-pathogenic *F. oxysporum*. FUCHS *et al*. (1997) attributed the bio-control activity of the non-pathogenic strain Fo47 to induced resistance in tomato, correlated with an increased activity of chitinase, β-1-3 glucanase and β-1-4 glucosidase. Finally, RECORBET *et al*. (1998) showed an overall increased activity of constitutive glycosidase isoforms in response to infection by *F. o.* f.sp. *lycopersici* that did not occur in roots colonized with non-pathogenic strains. These contrasted results obtained with the same strain of non-pathogenic *F. oxysporum* applied to tomato show that the biochemical response of the plant is still ignored and has to be accurately described before being able to compare resistance induced in tomato by non-pathogenic *F. oxysporum* to other plant – pathogen models where the cascade of biochemical events is better known.

Complementary modes of action

It must be strongly underlined that the different modes of action described above do not exclude each other. On the contrary a same non-pathogenic strain can express several modes of action. This is the case for the strain Fo47 for which several teams have reported the involvement of competition for nutrients in soil, competition for root colonization and induced local and systemic resistance. This is also the case for the non-pathogenic isolates C5 and C14 isolated by MANDEEL and BAKER (1991) and 618–12 (POSTMA & RATTINK 1992). One might expect that a strain expressing several modes of action would be more efficient and provide a more consistent control than a strain having a single mode of action.

CONCLUSION

This quick review of the studies dealing with the mechanisms involved in the biocontrol activity of non-pathogenic *F. oxysporum* and with their interactions with the plant showed that many different modes of action contribute to their efficacy. Based on this knowledge many teams have tried to develop a biological product to control Fusarium wilts. But one must admit that application of non-pathogenic *F. oxysporum* remains confidential. One of the main

reason to explain this failure is the lack of consistency of biological control. Therefore one must focus now on the conditions required to achieve successful and consistent control. Two lines of research must be followed, one dealing with the characterization of the saprophytic behavior of the non-pathogenic strains, the other with the environmental factors that control the biotic interactions.

The competitive ability of a non-pathogenic strain partly determines its capacity to establish in soil and in the plant rhizosphere and is probably involved in its capacity to colonize the root surface. But soil colonization depends also on the soil characteristics themselves, both biotic and abiotic. One must admit that ecological requirements of biological control strains have not extensively been studied. Similarly, colonization of the root surface and root tissues depends not only on the fungal strain but also on the plant species and plant cultivar. To our knowledge, the compatibility between strains of non-pathogenic *F. oxysporum* and the plant species has seldom been studied. Finally, when the main mode of action of a non-pathogenic strain is induction of systemic resistance, it is obvious that this phenomenon implies the physiological state of the plant and one can assume that depending on the environmental conditions, the plant will be more or less able to express its resistance to the pathogen, induced by the non-pathogenic strain. This lack of knowledge in these different fields might explain why some strains very effective under well controlled experimental conditions failed to give consistent control of the disease under field conditions.

To make biological control more consistent it is necessary to better know the determinism of both the pathogenicity, and the biocontrol capacity of strains of *Fusarium oxysporum*. One must admit that, despite the use of modern tools such as genetic engineering, we are far away from an acceptable understanding of the mechanisms by which a strain of *F. oxysporum* induces disease in a given plant species and protect another plant species against its specific pathogen. Many different properties are probably involved in the determinism of the antagonistic activity of non-pathogenic strains of *F. oxysporum*. Until today, it is very difficult to assess the relative importance of each mode of action. To accurately study the importance of these diverse modes of action an approach based on the selection of mutants affected in one or several traits would be necessary. Such an approach has been developed by TROUVELOT *et al*. (2002) using transposon mutagenesis. Mutants of Fo47 showing either an increased or a decreased biocontrol capacity have been selected, but it has not been possible to attribute these changes in the bio-control capacity to given phenotypic or genotypic trait of the mutants.

Much more research is therefore needed to achieve understanding of the determinism of the biocontrol capacity of non-pathogenic *F. oxysporum* and to determine the required conditions for successful control of Fusarium diseases through application of these non pathogenic strains.

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