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Nonpathogenic *Fusarium* as a Biological Control Agent

R. Kaur, J. Kaur and Rama S. Singh
Department of Plant Pathology, Punjab Agricultural University,
Ludhiana, India-141004, India

Abstract: *Fusarium oxysporum* is an important fungal group among the soil bone microflora. These strains are well-known for inducing wilt or root rots in important agricultural crops worldwide and some occur only as a saprophytes in rhizosphere of plants. There are certain strains which are nonpathogenic and protect plants from pathogenic strains. Based on phenotypic and genetic studies *F. oxysporum* showed a great diversity among its populations. The nonpathogenic strains, which were first isolated from suppressive soils strains showed several modes of action against pathogenic strains and thus exploited as biocontrol agents. These nonpathogenic strains suppress pathogens by competing for nutrients in the soil, reduce their chlamydospore germination, compete for infection sites on the root and induce systemic resistance in plant when invade host plant species before the pathogen. The nonpathogenic strains are formulated in talc and charcoal based media and commercial formulations are also available. These strains of *Fusarium* has been successfully combined with other biocontrol agents to obtain a effective biocontrol of plant pathogens. For application of nonpathogenic *Fusarium* under field condition some additional research is needed in several areas including: field studies and integration into production systems; risk assessment; and genetic improvement of biocontrol agents.

Key words: Agricultural crops, vegetables, pathogens, induce resistance, competition, biocontrol

INTRODUCTION

Fusarium oxysporum is one of the most common soil fungi in cultivated soil all over the world. It includes a large diversity of strains, all saprophytic, most parasitic (Garrett, 1970; Burgess, 1981). They are able to colonize to some extent plant tissues without inducing symptoms (Olivain and Alabouvette, 1997) and some are pathogenic inducing root rot or tracheomycosis. Many other strains can penetrate roots, but do not invade the vascular system or cause disease. The wilt-inducing strains of *F. oxysporum* cause a serious damage on many economically important agricultural crops (Benhamou *et al*., 1989; Hervas *et al*., 1998; Paul *et al*., 1999; Kaur, 2003). *Fusarium* wilt pathogens are very host specific. Based on their host plant species and plant cultivars, there are more than 53 forms, 117 formae specials and 29 varieties (Anonymous, 2010). Being a soil borne nature the management of *Fusarium* wilt is mainly through chemical soil fumigation like methyl bromide and use of resistant cultivars. But the use of soil fumigants has been banned because of their hazardous effects to human health and environment and methyl bromide is completely phased out. The most cost effective, environmentally safe method of control is the use of resistant cultivars, but over the time new races of the pathogen can develop which can infect resistant crop varieties. On the other hand breeding for resistance is possible only if a dominant gene is known and host is monoecious. In carnation, cyclamen, flax, no dominant gene is known and also the palm trees are dioecious (Fravel *et al*., 2003). Thus, the soil borne diseases are the target for biocontrol as convention control by agrochemical is problematic. With increasing awareness of deleterious effects of fungicides on the environment, growing interest in chemical free agricultural products and time consuming breeding programs, the biological control of plant pathogens have achieved a considerable attention.

Biological control of fusarial wilt diseases by nonpathogenic *Fusarium* have been reported in numerous crops in green house and field trials (Mandeel and Baker, 1991; Larkin *et al*., 1996; Larkin and Fravel, 1998; Katsube and Alasaka, 1997; Honda and Kawakub, 1998; Minuto *et al*., 1997a, b; Hervas *et al*., 1998; Fuch *et al*., 1999). Nonpathogenic *F. oxysporum* isolate Fo47 which was recovered from *Fusarium* wilt suppressive soils in France has been extensively studies for the control of fusarial wilt disease of several vegetable and flower crops (Alabouvette *et al*., 1993, 1998).

Corresponding Author: Ramandeep Kaur, Department of Plant Pathology and Crop Physiology,
Louisiana State University Agricultural Center, Baton Rouge, LA, USA-70803, USA

However, for biological control to be implemented commercially on practical level it is necessary to more fully understand the ecology of biocontrol agents and their interaction with host plant, pathogen and surrounding soil and rhizosphere microbial ecology (Cook, 1993). Ideally the antagonist must be ecologically fit to survive and function with the particular condition of the ecosystem. More over the antagonist must be present at adequate population level and be capable of effectively interacting with the pathogen or host plant to provide acceptable disease control.

Involvement of nonpathogenic *Fusarium* in soil supressiveness: Smith and Snyder (1971) and Toussoun (1975) gave the first evidence for a possible role of non-pathogenic *Fusarium* in suppressive soil and indicated that the soil supressive to *Fusarium* wilts supported a large population of nonpathogenic *Fusarium* sp. But it was confirmed by proving Koch's postulates i.e., by demonstration that the suppressiveness disappear after elimination of *Fusarium* by heat treatment and reappeared after reintroduction of the fungus in to the heat treated soil (Rouxel *et al*., 1979). Later on several studies clearly showed that nonpathogenic *Fusarium* sp. has a potential to suppress *Fusarium* wilts in different areas of the world (Schneider, 1984; Tamietti and Alabouvette, 1986; Paulitz *et al*., 1987; Tamietti and Pramotton, 1990; Larkin *et al*., 1993, 1996; Singh *et al*., 2002a, b). Although isolations of the *Fusarium* sp. from suppressive soils is an effective procedure for detecting strains able to control *Fusarium* diseases, the nonpathogenic strain of *F. oxysporum* selected by Ogawa and Komada (1984) and Postma and Rattink (1992) were obtained from stem of healthy plants. Whatever the origin the strains of *F. oxysporum* did not have the same ability to control the *Fusarium* wilts. Therefore screening procedures based on biotests have been developed to select the most efficient strain to control the disease. The plant pathogens controlled by nonpathogenic *Fusarium* has been listed in Table 1.

Isolation and identification

Selective medium: Conventionally, starch based media, such as PDA or malt agar are good substarte for the species of *Fusarium* and dematiaceous hypomycetes to grow rapidly and produce abundant aerial mycelia, but on these media sporulation is moderate, sometimes weak or absent. Media more selective for *Fusarium* and other dematiaceous hypomycetes have been formulated with high concentration of inhibitors or slowly assimilated carbon sources or both (Burgess and Liddell, 1983). Nash and Snyder (1962) described a selective media for *Fusarium* spp. which has peptone as base amended with 0.1% pentcholoronitrobenzene (PCNB) as a fungal inhibitor.

This medium is highly inhibitory, producing *Fusarium* colonies, which are scant and not the distinctive. PCNB (0.2%) has also been used in potato based medium supplemented with inorganic salts to isolate fusaria from barley and malt (Anonymous, 1981). Although these media are highly selective for some *Fusarium* species, they are markedly inhibitor of other with PCNB present in excess as a saturated solution. PCNB is undesirable as an constituents of media because it has been reported as potentially carcinogenic (Fairchild *et al*., 1977, 1992).

It has been shown that dicholoro (2, 6-dicholoro-4-nitroaniline) is a fungal inhibitor and effective substitute

Table 1: Diseases controlled by nonpathogenic *Fusarium oxysporum*

Disease	Causal organism	Reference
Fusarium wilt of gladiolus	*F. oxysporum* f.sp. *gladioli*	Magie (1980)
Fusarium wilt of cucumber	*F. oxysporum* f.sp. *cucumerianum*	Mandeel and Baker (1991), Paulitz *et al*. (1987)
Fusarium wilt of strawberry	*F.oxysporum* f.sp. *fragariae*	Tezuka and Makino (1991)
Fusarium wilt of carnation	*F. oxysporum* f.sp. *dianthi*	Minuto *et al*. (1997b), Lemanceau *et al*. (1993)
Fusarium wilt watermelon		Larkin *et al*. (1996)
Fusarium wilt of sweet potato	*F. oxysporum* f.sp. *batatas*	Ogawa *et al* (1996)
Fusarium wilt of spinach	*F. oxysporum* f.sp. *spinaciae*	Katsube and Alasaka (1997)
Fusarium wilt of rakkyo	*F. oxysporum* f.sp. *allii*	Honda and Kawakub (1998)
Fusarium wilt of raddish	*Fusarium oxysporum* f.sp. *raphani*	Toyota *et al*. (1995)
Fusarium wilt of Basil	*F. oxysporum* f.sp. *bascilis*	Minuto *et al*. (1997a), Larkin and Fravel (2002a, b)
Fusarium wilt of muskmelon	*F. oxysporum* f.sp. *cucumeranum*	Larkin and Fravel (1999a, b)
Fusarium wilt of chickpea	*F. oxysporum* f.sp. *ciceri*	Hervas *et al*. (1998), Paul *et al*. (1999), Singh *et al*. (2002b), Kaur (2003), Kaur *et al*. (2007b)
Fusarium wilt of tomato	*F. oxysporum* f.sp. *lycopersici*	Larkin and Fravel (1996), Fuch *et al*. (1999)
Fusarium wilt of pea	*F.oxysporum* f.sp. *pisi*	Benhamou and Grand (2001)
Fusarium wilt of flax	*F.oxysporum* f.sp. *lini*	Duijff *et al*. (1999)
Fusarium wilt of cyclamon	*F. oxysporum* f.sp. *cyclamonis*	Minuto *et al*. (1997b)
Pythium damping off	*Pythium ultimum*	Benhamou *et al*. (2002)
Fusarium wilt of banana	*F. oxysporum* f.sp. *cubense*	Gerlach *et al*. (1999), Ting *et al*. (2007)
Fusarium root and stem rot of *Cucumis sativus*	*F. oxysporum* f.sp. *radicis -cucumerinum*	Abeysinghe (2009)

for PCNB at very low concentrations (2 mg kg^{-1}). Burgess and Lidell (1983) described a modified Czapeck yeast extract medium supplemented with dicholoron (25 mg kg^{-1}) on which some fusaria produced distinctive colonies. With the reduced concentration of dicholoron selectivity of the medium was lost, which promoted the growth of *Aspergillus* and *Penicillium* species due the high nutrient status of czapeck yeast extract agar (Andrews and Pitt, 1986). Andrews and Pitt (1986) described the modified the Nash and Synder medium and developed a selective medium that contains 2 μg of dicholoron per mL, 200 μg of cholorophenicol per mL and 1.55 bacterological peptone (DCPA) for isolation of *Fusarium* spp. from cereals. The medium was designated as DCPA and showed selectivity against species of *Aspergillus*, *Penicillium*, *Cladosporium* and mucoraceous fungi. DCPA was evaluated for use as an enumeration medium and compared satisfactorily with dicholoron-rose bengal-cholorophenicol agar when both media were tested with a range of cereal samples. *Fusarium* species produced well formed colonies with good conidial production on DCPA medium permitting rapid identification of such isolates on this medium. Other media recommended for isolation of *Fusarium* are Carnation agar medium, potato dextrose medium, selective *Fusarium* medium, peptone PCNB agar, KCl agar, V8 Agar, Komada's medium, modified potato dextrose agar medium (Burgess *et al*., 1994).

Identification of *Fusarium oxysporum*: *Fusarium oxysporum* is highly variable in respect to colony morphology (Burgess *et al*., 1989). It produces floccose, sparse or abundant mycelium ranging in color from white to pale violet. Abundant pale orange or pale violet macroconidia are produced in a central spore mass in some isolates. Small pale brown, blue to blue-black or violet sclerotia are present, sometimes abundant, in a minority of isolates. *Fusarium oxysporum* usually produces a pale to dark violet or dark magenta pigment in the agar but some isolates do not produce any pigment. Some isolates of *F. oxysporum* degenerate readily to the pionnotal form or to a flat wet mycelial colony. Macroconidia are formed in pale orange, usually abundant, sporodochia. The macroconidia are short to medium in length, falcate to almost straight, thin walled and usually three septate. They tend to be somewhat pointed or tapered at each end. The apical cell is short and is slightly hooked. in some isolates. The base of the basal cell is notched or foot-shaped. The macroconidia are formed from monophialides on branched conidiophores in sporodochia and to a minor extent from monophialides on hyphae. Microconidia are usually formed abundantly in false-heads on short monophialides on hyphae. The microconidia are usually nonseptate and are oval, elliptical or reniform (kidney-shaped). Some cultures do not form abundant microconidia. Chlamydospores are formed abundantly in most isolates, especially saprophytic clones from soil, but may be slow (3 to 6 weeks) to form in some isolates. They are more obvious in hyphae on the surface of the agar of the CLA plate. The formation of microconidia in false-heads on short phialides formed on the hyphae, chlamydospores and the shape of the macroconidia and microconidia all help to distinguish *F. oxysporum*. Isolates of *F. oxysporum* can be difficult to distinguish from *F. solani* and *F. subglutinans*. A key feature of *F. solani* is the formation of microconidia in false-heads on very long monophialides formed on the hyphae. *Fusarium subglutinans* is distinguished by the formation of microconidia from polyphialides and the absence of chlamydospores. Polyphialides are, however, difficult to find in some isolates of *F. subglutinans*. Thus all characters need to be assessed carefully when identifying these species.

Fusarium oxysporum is common in cultivated soils of temperate and tropical areas of eastern Australia and is common in soils from tropical, subtropical and temperate forests (Summerell *et al*., 1993). It is also common in improved-pasture soils of the temperate areas but was not common in grassland soils of Western Queensland (Burgess and Summerell, 1992) and was not isolated from soil from the Simpson Desert, Australia.

Fusarium oxysporum included many representatives which are pathogenic to plants causing vascular wilt diseases (Beckman, 1987), damping-off problems (Nelson *et al*., 1983) and crown and root rots (Jarvis and Shoemaker, 1978). Isolates which cause wilt diseases are usually host specific and over 100 formae speciales and races have been shown to exist. Wilt diseases are a major problem in many vegetable and ornamental crops, bananas and palms (Nelson *et al*., 1981; Summerell and Rugg, 1991). Cereals and grasses are apparently unaffected by *F. oxysporum*.

Identification can be done on the basis of shape of macroconidia, presence and absence and shape of micro conidia, chlamydospore formation, colony diameter, colony morphology etc.

Selection of nonpathogenic *Fusarium*: The minor differences in morphology of different species makes the identification of *Fusarium* spp. from soil, a difficult task. and also the differences in cultural conditions can cause variations in *Fusarium* spp. (Doohan, 1998). The nonpathogenic strains of *F. oxysporum* were first isolated

from natural *Fusarium* suppressive soils (Smith and Snyder, 1971; Alabouvette, 1990; Postma and Rattink, 1992; Larkin *et al*., 1996; Edel *et al*., 1997). These are also found from the plant rhizosphere and rhizoplane, without inducing any symptoms (Elias *et al*., 1991; Olivain and Alabouvette, 1999; Kaur, 2003; Kaur *et al*., 2007a). It is occasionally difficult to distinguish *F. oxysporum* from several other species belonging to the sections Elegans and Liseola (Fravel *et al*., 2003). The plant pathogenic, saprophytic and biocontrol strains of *F. oxysporum* are morphologically impossible to differentiate (Fravel *et al*., 2003). For selecting pathogenic and nonpathogenic isolates of *F. oxysporum* a bioassay method was suggested by Robert and Kraft (1971). In this three week old seedlings of test crop, grown on sterilized soil were aseptically transferred into different test tubes containing the spore suspension (1×10^4 cfu mL^{-1}) of different isolates of *F. oxysporum*. The tubes kept on a rotary shaker at 50 rpm for 24 h for development of disease. Based on this technique, Kaur (2003) isolated nonpathogenic *Fusarium* collected from rhizosphere and rhizoplane of chickpea in India and categorized isolates of *Fusarium* as nonpathogenic: no wilt symptom appeared upto seven days of incubation; least pathogenic;- wilt symptoms developed after 6-7 days and <33% wilting occurred; moderately pathogenic-wilt symptoms developed after 4-5 days and <66% wilting occurred; and highly pathogenic; -symptoms appeared after two days and plants wilted on 4th day and >67% wilting occurred. (Kaur, 2003; Kaur *et al*., 2007a, b). Besides this a classification system using Vegetative Compatibility Groups (VCG) among the *F. oxysporum* strains was anticipated (Puhalla, 1985) based on pairing nitrate nonutilizing mutants. This system was later on modified by standardization of numbering of VCG (Kistler *et al*., 1998). In this system some *formae speciales* correspond to a single VCG, while others include several VCGs. Among nonpathogenic populations, many isolates are single member VCGs and some are even self-incompatible (Gordon and Okamoto, 1992; Kondo *et al*., 1997; Steinberg *et al*., 1997; Fravel *et al*., 2003). Thus, this system was useful for the determination of VCG but cannot be used as a universal tool to identify *formae specials* or nonpathogenic isolate. (Fravel *et al*., 2003). These difficulties have been overcome by use of molecular tools like ITS-RFLP (Edel *et al*., 1995), IGS-RFLP (Appel and Gordon, 1994) which were developed to support morphological identifications different *Fusarium* sp. Edel *et al*. (1995) developed a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method targeting a fragment of the ribosomal rDNA that includes the internal transcribed spacer (ITS) region for the identification of *Fusarium* species. Edel *et al*. (2000) also developed an rDNA targeted oligonucleotide probe and PCR assay specific for *F. oxysporum*. Mishra *et al*. (2003) developed a PCR-based assay for rapid identification of some *Fusarium* species.This technique is based on the ITS region of the rDNA. Nel *et al*. (2006) designed a PCR-based RFLP analysis of the intergenic spacer region of the ribosomal RNA operon to characterize the nonpathogens. The PCR-RFLP analysis with species-specific primers FOF1 and FOR1, was conducted to confirm the identity of nonpathogenic *F. oxysporum* isolates from pathogenic *F. oxysporum*

Mode of action of nonpathogenic *Fusarium*: The two primary mechanism of action associated with nonpathogenic *Fusarium* spp. are: induced systemic resistance (ISR) and competition: The competition can be further categorized in to; saprophytic competition for nutrients in the soil and rhizosphere and parasitic competition for infection sites on the roots. The mechanism of action of antagonist can provide the much insight in to where and when the interaction occurs, the way pathogen is affected and how the antagonist needs to be implemented.

Induced Systemic Resistance (ISR): ISR is a systemic induction of defense responses with in the plant triggered by some biotic and abiotic stress inducing agents (Komada, 1975). In case of *Fusarium* wilts, Matta (1989) observed a phenomenon of cross protection, when pre-inoculation of a plant with an incompatible strain of *Fusarium* sp. resulted in the mitigation of symptoms when plant was later inoculated with a compatible strain. The induced resistance has been considered as a mechanism that could be responsible for disease control induced by nonpathogenic *F. oxysporum* (Table 2). Mandeel and Baker (1991) have shown that a nonpathogenic strain applied on some roots of the plant can delay disease symptom induced by a pathogen applied to other roots or in the stem of the plants. Tamietti *et al*. (1993) demonstrated that tomato plants grown in suppressive soil showed the higher content in several hydrolytic enzymes related to the PR protein, the same enzymatic activities were increased in plants grown in sterilized soil artificially infested with strains of nonpathogenic *F. oxysporum* that induce control of the disease. Hillocks (1986) working with induced resistance to *Fusarium* wilt of cotton concluded that mechanism was vascular occlusion induced by a nonpathogenic strain of *F. oxysporum* that limited the movement and reduced populations of the

Table 2: Nonpathogenic *Fusarium* species responsible for induction of induced resistance

Plant sp.	Nonpathogenic strain	Challenging pathogen	Reference
Cucumber	*F. oxysporum*	*F.o.* f.sp. *cucumerianum*	Mandeel and Baker (1991)
Tomato	*F. oxysporum*	*F.o.* f.sp. *lycopersici*	Kroon *et al.* (1992), Olivain *et al.* (1995), Fuchs *et al.* (1997)
Tomato	*F. oxysporum* CS1 CS20	*F.o.* f.sp. *lycopersici*	Larkin and Fravel (1999a, b)
Pea	*F. oxysporum*	*F.o.* f.sp. *pisi*	Benhamou and Garand (2001)
Cucumber	*P.putida* A 12 and NIR nonpathogenic *Fusarium* sp.	*F.o.* f.sp. *cucumerianum*	Park *et al.* (1988)
Watermelon		*F.o.* f.sp. *cucumerianum*	Biles and Martyn (1989), Larkin and Fravel (2002a, b)
Ipomea tricolor	*F. oxysporum* 1012	*F. oxysporum*	Shimizu *et al.* (2000)

pathogen within vascular tissue. Duijff *et al.* (1998) reported that the suppression of *Fusarium* wilts achieved by nonpathogenic *F. oxysporum* Fo47 appeared to be systemic induced resistance. This induced resistance could be related to the accumulation of PR-1 and chitanases. Induced resistance in tomato plants against *Fusarium* wilts raised by nonpathogenic *Fusarium* sp. isolated from roots of faba beans in southern Sweden (Attitalla *et al*., 1998). ISR was observed in specific nonpathogenic strain of *Fusarium* sp. isolated from wilt suppressive soils for the control of *Fusarium* wilt diseases of tomatoes, watermelon and muskmelon (Larkin and Fravel, 1999a). These antagonists were effective at low inoculum density at high pathogen densities. In addition production of some antifungal substances was also observed in response to the nonpathogenic *Fusarium* sp. inoculation induced resistance in pigeon pea seedlings against *F. udum* infection (Chakraborty and Gupta, 1998). A thermolabile chemical component produced by nonpathogenic isolates of *Fusarium* (*F. oxysporum* 1012) was associated with the induction of resistance against fusarial wilt of *Ipomea tricolor* (Shimizu *et al*., 2000). Other reports of non-pathogenic *F. oxysporum* reducing disease via a host-mediated mechanism have been studied by Huertas-Gonzalez *et al.* (1999). Although these results showed that induction of resistance could be one of the modes of action of nonpathogenic *F. oxysporum*, much more research is needed to clearly identify the molecules responsible for induced resistance. Sometimes when the nonpathogenic strain is physically separated from the pathogenic strain, is less affected than control achieved when the two strains are introduced together in the soil, which indicates the role for other mechanisms of action, especially mechanism of competition between pathogenic and nonpathogenic *F. oxysporum.*

Competition

Saprophytic competition for nutrients in soil and rhizosphere: It was assumed that for soil suppresiveness is governed by competition for carbon. Alabouvette *et al.* (1985) reported that the total biomass was generally greater and more active in suppressive than in conducive soil and strains of *F. oxysporum* differ in their ability to utilize glucose. efficiently. Lemanceau *et al.* (1992) established that competition for carbon was one of the mechanisms by which the nonpathogenic strain Fo47, *in vitro* inhibited the growth of *F.oxysporum* f.sp. *dianthi* strain WCS-816. These results are in accordance with those obtained by Couteaudier and Alabouvette (1990) demonstrating that different strains of nonpathogenic *F. oxysporum* did not have the same ability to grow in soil enriched with glucose nor the ability to compete with pathogenic strain to colonize a disinfested soil. A mathematics model was used to calculate index characteristics of competitive ability of several strains of *F. oxysporum* growing together in steamed soil. A strong correlation was observed between the competitive index, the growth yield in disinfested soil enriched with glucose and the ability of the strains to control *Fusarium* wilts of flax (Alabouvette and Couteaudier, 1992). However, chlamydospore germination of two pathogens, *F. oxysporum* f.sp. *lycopersicum* and *F. oxysporum* f.sp.*bascilis* in soil at increasing concentration of glucose was inhibited by three nonpathogenic Fusaria (CS1, CS20 and Fo47) (Larkin and Fravel, 1999b), indicating the saprophytic competition for nutrients as a mechanism of action of Fo47. Competition for nutrients as one of the mode of action of other isolates of nonpathogenic *F. oxysporum* has also been observed in such as strain 618.12 (Postma and Rattink, 1992) and strains C5 and C14 (Mandeel and Baker, 1991; Paul *et al*., 1999).

Competition for infection sites at the root surface: There are infinite number of infection sites that are protected by increasing the inoculum density of nonpathogenic strains (Mandeel and Baker, 1991). Indeed, control of the disease not necessarily a high inoculum density of nonpathogenic strain but a high ratio of nonpathogen to pathogen strain. Duijff *et al.* (1999) found that strain Fo47 was effectively competing with the pathogen at the apex of the flax root and reduced both the colonization rate by the pathogen and activity of the pathogen in the flex root tissues. The extensive colonization by nonpathogenic strain (70T01) of *Fusarium* prior to invasion by the pathogen is likely of great importance in the direct interaction with the pathogen for the reduction of pathogen infection and

thus for disease reduction (Bao and Lazarovits, 2001). A three phase mechanism was associated with biological control of *Fusarium* wilt of cucumber by two nonpathogenic strains of *F. oxysporum* (C5 and C14) (Mandeel and Baker, 1991). There was a significant reduction in germination of chlamydospores of *F. oxysporum cucumerinum* in rhizospheres of cucumbers infested with C5 or C14. Competition for infection sites with *F. oxysporum* f.sp. *cucmerinum* was demonstrated in soil infested with C14 but not with C5. Enhanced systemic resistance of the host to inoculation with pathogen *F. oxysporum* f.sp. *cucumerinum* (Mandeel and Baker, 1991). Several other studies were also conducted to demonstrate that pathogenic and nonpathogenic strains were competing for root colonization (Ogawa and Komada, 1984; Nagao *et al*., 1990; Eparvier and Alabouvette, 1994; Postma and Luttikholt, 1996; Hervas *et al*., 1997; Benhamou and Garand, 2001).

Histological and cytological studies in response to colonisation by nonpathogenic *F. oxysporum*: The cytology of root infection by nonpathogenic *Fusarium* with that associated to a pathogenic *Fusarium* strain was first studied by Benhamou and Garand (2001). They found the nonpathogenic strain grew actively at the root surface and colonized a number of epidermal and cortical cells whereas in roots inculcated with pathogenic strain, the fungus multiplied a massive elaboration of hemispherical wall appositions and deposition of an electron-opaque material frequently encircling pathogen hyphae abundantly through much of the tissues. The host roots were signaled to defend themselves through the rapid stimulation of a general cascade of nonspecific defense responses. In other studies the nonpathogenic *Fusarium* displayed the ability to colonize the outer root tissues without inducing cell damage, which is known to occur in several host-*Fusarium* sp. interactions (Benhamou and Theriault, 1992). This property implies that at least small amounts of cell wall hydrolytic enzymes, such as pectinases and cellulases, were produced by nonpathogenic *Fusarium* to locally infringe the host cell walls, thus, facilitating spread into the root tissues. However, the regular pattern of cellulose distribution over host cell walls adjacent to invaded areas was taken as an indication that cell wall-degrading enzymes were slightly produced inside the plant (Benhamou and Garand, 2001). Synthesis of extracellular lytic enzymes by nonpathogenic *Fusarium* strains has not been reported, although the possibility that these fungi may produce pectinases and cellulases as part of their enzymatic arsenal appears realistic. Pectin hydrolysis is one of the main mechanisms involved in root colonization by nonpathogenic strains (Benhamou and Theriault, 1992), evidenced by the presence of the fungus in a large number of intercellular spaces. Thus the relationship established between the host plant and nonpathogenic *F. oxysporum* appears to follow a well-defined scheme of events including proliferation along the elongating root and local penetration of the epidermis resulting in the release of pectic fragments in turn, may act as elicitors of the plant defenses (Benhamou *et al*., 1996). Wilson (1995) called nonpathogenic strains as endophytes because they establish in cortical tissues, without causing disease symptoms. Mandeel and Baker (1991) observed that cytochemical analysis of the pattern of chitin distribution revealed a marked decrease in the amount of chitin over cell walls of nonpathogenic *Fusarium*, especially at sites where the fungus was closely appressed against the host cell wall. Collectively, these results indicate that the root cells were signaled to produce chitinases that likely accumulated extracellularly (Mandeel and Baker, 1991), Understanding the molecular mechanisms regulatory circuit involved in plant gene root infection by nonpathogenic fungal represents a major challenge for future research (Benhamou and Garand, 2001).

Formulations of nonpathogenic *F. oxysporum*: Once a biocontrol agent is selected on the basis of their potentiality *in vitro* and *in vivo* production of their biomass and suitable formulation becomes the major concern (Lumsden and Lewis, 1989). Successful biocontrol depends on having the biocontrol agent delivered to the right place, at the right time, in the appropriate physiological state. In addition to these considerations, application must be compatible with the production system (Minuto *et al*., 1997a, b; Fravel *et al*., 2003). Minuto *et al*. (1997b) have compared different strains of nonpathogenic *F. oxysporum* and different commercial formulations of these strains used to control *Fusarium* wilt of basil. The formulation can be applied as; seed treatment and soil treatment. For formulation of antagonist biomass in suitable form is another important step for the success of biological control. The formulation must be easy to handle and deliver to the site of action. The talc, charcoal and chem-sorb are commonly used as inert carriers in preparing the formulation of nonpathogenic *Fusarium* (Steinberg *et al*., 1997, 1999: Kaur, 2003). The population kinetics and the biological efficacy of several formulations of Fo47 were compared and evaluated in greenhouses and fields (Steinberg *et al*., 1997). A formulation made of microgranules enriched with food base provided a better survival and a better biocontrol efficacy than the traditional talcum formulation used in the laboratory. The amendment of non-pathogenic

Fusarium formulations with farm yard manure also enhanced the biocontrol potentiality of non-pathogenic *Fusarium* against chickpea wilt (Singh *et al*., 2002a).

COMMERCIAL FORMULATIONS OF NONPATHOGENIC *FUSARIUM*

Biofox C:

- **Target pathogen:** *F. oxysporum* and *F. moniliformae*
- **Crops:** Basil, carnation, cyclamon, tomato
- **Formulation:** Dust or alginate granules
- **Method of application:** Seed treatment or soil incorporation
- **Country registered:** Italy
- **Manufacturers and suppliers:** SIAPA

Fusaclean:

- **Target pathogen:** *F. oxysporum*
- **Crops:** Asparagus, basil, carnation, cyclamon, tomato, Gerbera
- **Formulation:** Liquid formulation
- **Method of application:** Incorporate into potting mixture; in rows
- **Country registered:** France
- **Manufacturers and suppliers:** Natural plant protection

Combining nonpathogenic *Fusarium* with other microorganisms: The nonpathogenic *Fusraium* has been successfully combined with *Pseudomonas* sp. to obtain a effective biocontrol of plant pathogens. Couteaudier and Alabouvette (1990) suggested that the efficacy of non-pathogenic *F. oxysporum* strains in controlling *Fusarium* wilt is related to their ability to compete for carbon. Numerous studies have established that fluorescent *Pseudomonas* sp. are efficient competitors for ferric iron (Kloepper *et al*., 1980; Larkin and Fravel, 1998). Their siderosphores, called pyoverdines or pseudobactins, have a high affinity for iron (Bakker *et al*., 1988). Siderophore- mediated competition for iron was demonstrated or postulated to be responsible for suppression of disease development of several soil borne pathogens, including *Fusarium* wilt, by strains of fluorescent *Pseudomonas* sp. The greater efficacy in disease suppression by the association of non-pathogenic *F. oxysporum* and fluorescent *Pseudmonas* spp. could be due to the combination of carbon and iron competition. Lemanceau *et al*. (1992) observed that nonpathogenic *F. oxysporum* Fo47b10 combined with *Pseudomonas putida* WCS358 competently suppressed *Fusarium* wilt of carnations grown in soil less culture. This suppression with this combination was considerably higher than that obtained by use of either antagonistic micro-organism alone. The increased suppression obtained by Fo47b10 combined with WCS358 only occurred when Fo47b10 was introduced at a density high enough (at least 10 times higher than that of the pathogen) to be efficient on its own. The *P. putida* isolate WCS358 had no effect on disease severity when inoculated on its own but significantly improved the control achieved with nonpathogenic *F. oxysporum* Fo47b10. In contrast, a siderophore-negative mutant of ICS358 had no effect on disease severity even in the presence of Fo47b10. Since the densities of both bacterial strains at the root level were similar, the difference between the wild-type yCS358 and the siderophore-negative mutant with regard to the control of *Fusarium* wilt was related to the production of pseudobactin 358. The production of pseudobactin 358 appeared to be responsible for the increased suppression by Fo47b10 combined. Competition for nutrients was proposed as a mechanism for suppression of *Fusarium* diseases by both non-pathogenic *F. oxysporum* and fluorescent *Pseudomonas* sp. Lemanceau *et al*. (1992) suggested that pseudobactin 358 production by *P. putida* WCS358 was responsible for the improved biological control of *Fusarium* wilt achieved by association of non-pathogenic *F. oxysporum* Fo47b10 and *P. putida* WCS358 compared with the separate application of each antagonistic organism. These results suggest that pseudobactin-mediated competition for iron increases the efficacy of the antagonistic activity of nonpathogenic *F. oxysporum* Fo47b10 against pathogenic *F. oxysporum*. Similar results were obtained with combined application of an isolate of nonpathogenic *F. oysporum* and a isolate of fluorescent *Pseudomonas* against *Fusarium* wilt of chickpea in India (Kaur *et al*., 2007a, b). Ting *et al*. (2007) studied the efficacy of *F. oxysporum* isolate UPM31P1 and *Serratia marcescens* isolate UPM39B3 in suppressing *Fusarium* wilt incidence in Pisang Berangan. In glasshouse trials the treatments with UPM31P1 singly and in combination with UPM39B3 were effective in delaying the onset of *Fusarium* wilt symptoms and in reducing disease severity and incidence. Under field conditions UPM31P1 applied singly and in combination with UPM39B3 was able to suppress wilt incidence in plants up until week 13 after planting. This amounted to a 6-week delay in the appearance of symptoms compared to untreated control plants. Treatment with UPM31P1 and UPM39B3 initially suppress the wilt incidence and encourage vegetative growth. Elmer (2008) studied the

biocontrol potential of two strains of nonpathogenic *F. oxysporum* strains (CS-20 and CWB318) against *Fusarium* crown rot on asparagus when combined with and without NaCl. In the greenhouse and field, there were no interactions between the treatments and NaCl on root lesions, root growth or yield. The nonpathogenic *Fusarium* strain CS-20 increased root growth. Fravel *et al.* (2005) assessed the compatibility of strain CS-20 with seven fungicides recommended for tomato. All the tested fungicides tested did not kill strain CS-20 at the concentrations tested in the *in vitro* experiment. Azoxystrobin (Quadris) and chlorothalonil (Bravo) were most toxic to strain CS-20 and significantly reduced growth rate and final colony size at 10 ppm a.i. or greater concentrations compared to growth on unamended medium. Thiram (thiram), Mefenoxam+ chlorothalonil (Ridomil Gold Bravo) significantly reduced final colony size at 30 ppm and 50 ppm or greater respectively. Mancozeb (Manzate) and mancozeb+copper (Mankocide) reduced final colony size only at 100 ppm, while mefenoxam (Ridomil Gold) and mefenoxam+ copper (Ridomil Gold Copper) did not affect growth of strain CS-20.

CONCLUSIONS

Biological control of *Fusarium* wilt diseases has become an increasingly popular disease management consideration in recent years, given its environmentally friendly nature and the discovery of novel mechanisms of plant protection associated with certain microorganisms. From a crop protection point of view, nonpathogenic strains of *F. oxysporum* represent a key component of the soil microbiota in soils suppressive to *Fusarium* wilt. Better knowledge of the mechanisms involved in the protection of plants by biocontrol agents is a requirement to the development of successful biocontrol strategies for use under commercial conditions. Also, before planning the large-scale use of nonpathogenic strains of *F. oxysporum* as biocontrol agents of *Fusarium* wilt, their behaviour and potential impact on soil ecosystems should be carefully studied as part of risk assessment.

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