Symbiosis, 9 (1990) 343–354
Balaban Publishers, Philadelphia/Rehovot

Colonization of Sterilized Soil and Flax Roots by Strains of *Fusarium Oxysporum* and *Fusarium Solani**

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Abstract

Several studies showed that nonpathogenic strains of *Fusarium* competing either saprophytically or parasitically with the pathogen could be used to control Fusarium-wilts of plants. Nine strains of *F. oxysporum* and *F. solani* were compared for their specific ability to colonize a sterilized soil and the cortex of flax roots growing in the soil precolonized by these strains.

Introduced at the initial concentration of 1.0×10^3 CFU/g into the steamed soil, the nine strains of *Fusarium* grew and reached a plateau after 2 weeks. The inoculum densities at the plateau were statistically different showing that these strains did not have the same ability to colonize a sterilized soil. There were clear differences in the ability of these strains to colonize the roots of flax growing through the soil. The percentage of root fragments colonized by *Fusarium* was not correlated with the population density in soil, showing that the ability to colonize soil and the ability to colonize the root are different properties of the same strains. The two nonpathogenic strains that are the best in reducing the percentages of plants infected by the pathogen also were the best root colonizers. But these results did not allow us to draw any conclusions about the existence of a relationship between the ability of the strains to colonize the root and their ability to induce biological control of wilts.

Introduction

Several studies indicated that Fusarium-wilt suppressive soils contained large population densities of nonpathogenic *Fusarium* (Smith and Snyder 1972; Toussoun 1975; Louvet et al., 1976). Moreover, it was demonstrated that introductions of nonpathogenic *Fusarium* spp. into conducive soils or soilless cropping substrates induced biological control of *Fusarium*-wilts (Rouxel et al., 1979; Schneider 1984; Tramier et al., 1984; Garibaldi et al., 1987; Alabouvette et al., 1987a, b; Paulitz et al.,

*Reviewed

1987). These observations led to the conclusion that some strains of nonpathogenic *Fusarium* have the ability to limit the activity of pathogenic strains of *Fusarium oxysporum* responsible for wilts.

Different hypotheses were proposed to describe the interaction between pathogenic and nonpathogenic *Fusarium* resulting in decreased wilt incidence. Comparing the level of soil and root colonization by *Fusarium* in a suppressive and a conducive soil, Alabouvette et al., (1984) proposed that pathogenic and nonpathogenic *Fusarium* were competing saprophytically in soil, in the vicinity of the roots. Following a similar approach, Schneider (1984) concluded that some strains of nonpathogenic *Fusarium* were able to compete with the pathogenic *Fusarium* at the root surface for infection sites. This author referred to this mechanism as "parasitic competition" in opposition to "saprophytic competition" in soil. Finally, Ogawa and Komada (1983) selected a strain of nonpathogenic *Fusarium* able to colonize the vascular tissues of sweet potato and to induce cross-protection against the pathogen. These three different modes of action were not mutually exclusive; they all could probably contribute to control of Fusarium-wilts of plants.

Until now the nonpathogenic strains of *Fusarium* used for biological control were selected by means of a bioassay that did not take into account the mode of action of the strains (Corman et al., 1986). For a better understanding of the mechanisms of biological control, it is necessary to elucidate the mode of action of the selected strains of nonpathogenic *Fusarium*. The aim of this study was to compare the ability of strains of *F. oxysporum* and *F. solani* to colonize a sterilized soil or flax root, to determine if a correlation exists between the efficiency of the strains to control fusarium-wilt of flax in sterilized soil and their ability to colonize the soil or the roots of the plant.

Material and Methods

Fusarium Strains

Strains of *Fusarium* used throughout this work were: four nonpathogenic *F. oxysporum*, Fo20, Fo34, Fo47 and Fo50 and two nonpathogenic *F. solani*, Fs2, and Fs59, previously isolated from the suppressive soil of Châteaurenard; two *F. oxysporum* pathogenic on plants other than flax, Fol8, and Fo44 corresponding to f.sp. *lycopersici* race O and to f.sp. *dianthi*, respectively; and one pathogenic strain of *F. solani* f.sp. *phaseoli*. For long term storage all isolates were kept at 4°C on P.D.A., the cultures being transferred once a year. To prepare inoculum, the *Fusarium* strains were grown in shake-culture on nutrient broth (malt extract 10g in 1 l. of distilled water). After 10 days, the growth medium was removed by centrifugation at 2000g the conidia washed in sterile water and mixed with talc (1 ml of conidia suspension in 2 g of talc). The mixture was dried at 18–20°C by forced air, sieved through a mesh of 200 μm, and stored at room temperature until used in experiments. The population density of

Fusarium in talc was estimated by culture on malt agar following the suspension dilution technique. Three suspensions were made by addition of 1 g of talc in 99 ml of sterile water, and 10 Petri dishes were poured with 1 ml of the suitable dilution to obtain 20–60 colonies per dish. The population density resulted from the mean of these 3 replicates of 10 Petri dishes.

Soil.

Soil samples used in these experiments came from an experimental plot of the research center in Dijon. Aluminium jars were filled with 300 g of dry soil sieved through a mesh of 2 mm. The water content was adjusted at 12% (d.w.) and the samples were autoclaved at 120°C for 30 minutes. This was repeated 3 times at 48 hours intervals. To facilitate mixing of inoculum with soil, the last treatment was followed in the autoclave, by a drying process which reduced the water content to 7%. Microbiological analyses failed to detect any bacteria during the first 5 days after sterilization.

Soil colonization by Fusarium

The ability of *Fusarium* strains to colonize sterilized soil was assessed by determining the population density reached 2 weeks after soil infestation at an initial inoculum density of 1×10^3 CFU/g soil, added with 5g of talc. Soil water content was adjusted to 18% corresponding to a water-potential of 0.1 MPa. The jars were incubated at 25°C for 2 weeks, and the samples were homogenized twice by shaking the jars. Then the inoculum density of *Fusarium* was assessed using the soil-plate dilution technique and culture on malt agar. Ten replicates plates were made from each soil sample and 3 soil samples per treatment. After logarithmic transformation the data were compared by analysis of variance followed by the test of Newmann and Keuls ($P = 0.05$). After 2 weeks of colonization by the strains of *Fusarium* spp., the soil was distributed in sterile glass tubes (20 mm × 120 mm) previously partly filled with 8 cm of sterile Perlite. Seven g of soil corresponding to a 2 cm layer were covered with a 1-cm-layer of sterile Perlite. The tubes were irrigated daily with sterile water to maintain the soil at field capacity. After 21 and 35 days of incubation in the presence or absence of flax roots (as described below), the soil was pushed out of the tube and population density was determined as described above. There were 3 tubes analysed per treatment.

Root colonization by Fusarium

The ability of strains to colonize roots of flax was assessed by use of a technique adapted from Wilkinson et al., (1985). Seven gram of soil, previously colonized by *Fusarium* for 2 weeks, were deposited in sterile glass tubes, (ϕ 20 mm × 120 mm) previously partly-filled with 8 cm of sterile Perlite as described above. Two seeds of

flax (*Linum usitatissimum*), cultivar Regina, were surface sterilized in a 2% solution of NaOCl, laid at the top of the soil, and covered with a 1 cm layer of sterile Perlite.

After emergence, only one seedling per tube was kept. The tubes were irrigated daily with sterile water to maintain the soil at field capacity. After 21 and 35 days of culture at 25°C, the plants were removed, and the main root was washed out of the soil. The root was surface disinfected for 3 minutes in a 2% solution of NaOC1 and rinsed three times in sterile water, as described by Schneider (1984). Then, the 2 cm root segments that were growing into the soil were cut into 1 mm pieces and transferred to malt extract agar. *Fusarium* colonies arising from the root fragments were determined after 5 to 7 days of culture at 25°C. For each treatment there were 10 replicated tubes. The

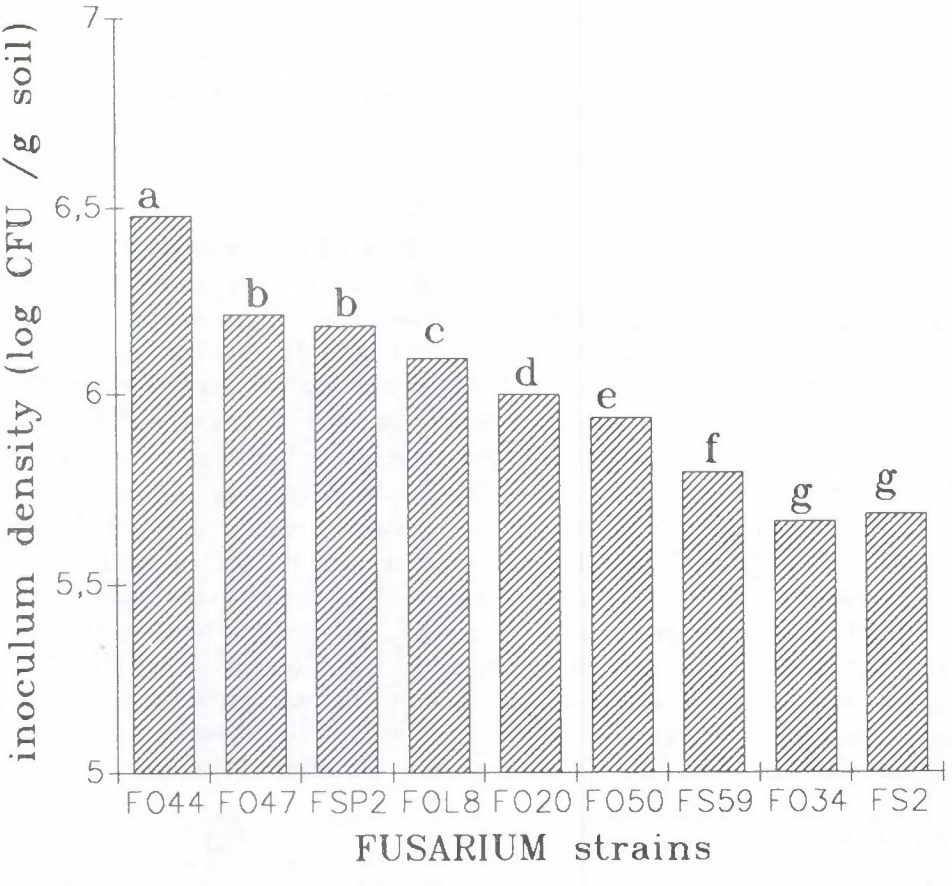

Figure 1. Inoculum density (log CFU/g soil) of nine strains of *Fusarium* added to sterilized soil at the initial density of 1.0×10^3 CFU/g soil. Inoculum density was determined after 2 weeks of incubation. Columns with a common letter are not significantly different by Newmann and Keuls test ($p = 0.05$).

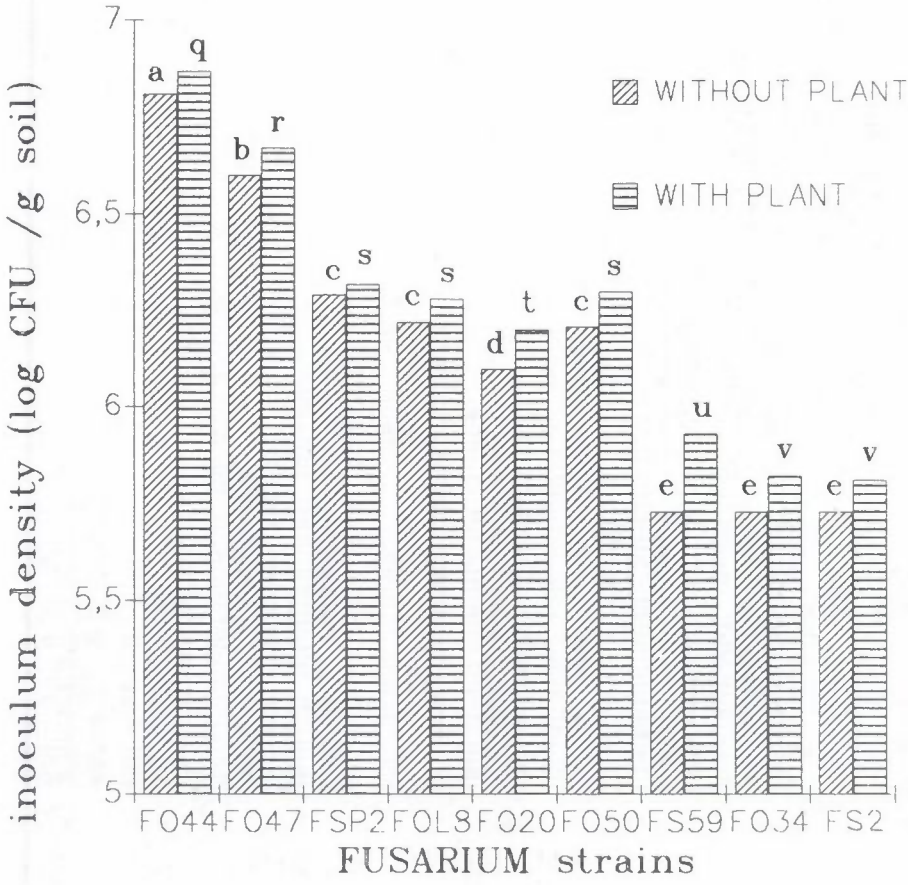

Figure 2. Inoculum density (log CFU/g soil) of nine strains of *Fusarium* in soil after 21 days, with and without flax plants. Soil was precolonized for 2 weeks by the strains of *Fusarium* initially added to sterilized soil at 1.0×10^3 CFU/g soil (cf. Fig. 1). Population densities in the presence of flax roots are significantly higher than population densities in the absence of roots (F. test significant at $p = 0.05$). In each case, absence or presence of roots, columns with common letters are not significantly different by Newmann and Keuls test ($p = 0.05$).

percentages of root colonization (number of segments colonized by *Fusarium* spp/total number of segments × 100) were statistically analysed after angular transformation. All the experiments were repeated with similar results.

Results

Ability of the strains of Fusarium *to colonize a steamed soil*

Figure 1 shows the inoculum density attained by the populations of *Fusarium* 2 weeks after their introduction in sterilized soil at 1.0×10^3 CFU/g soil. All the strains

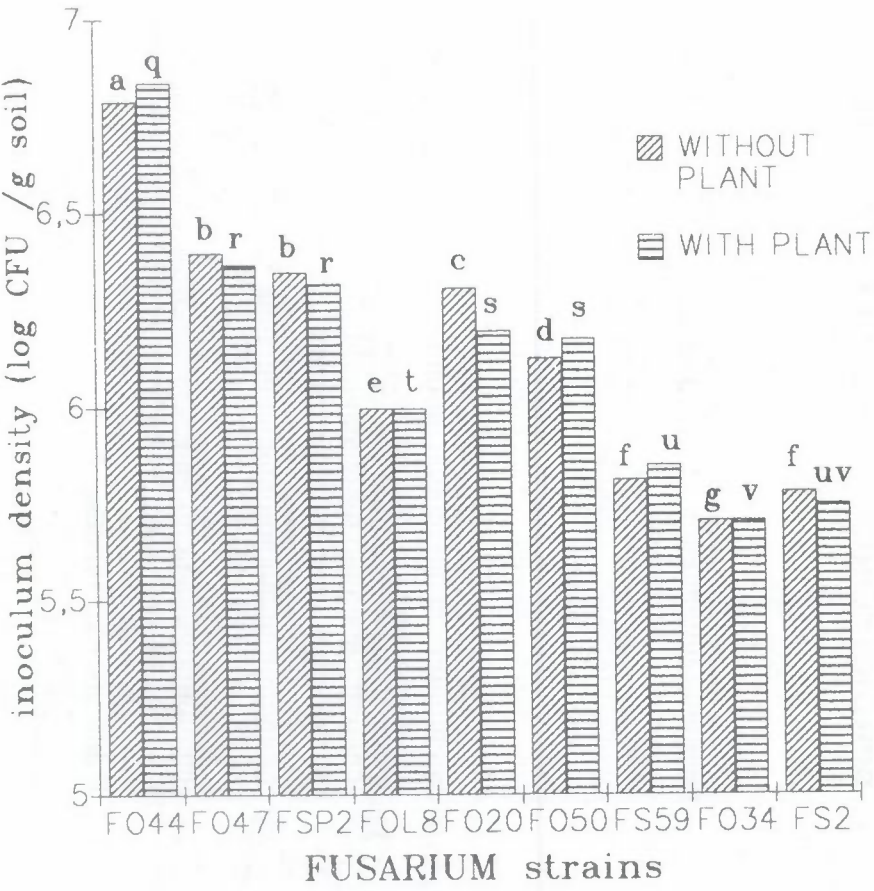

Figure 3. Inoculum density (log CFU/g soil) of nine strains of *Fusarium* in soil after 35 days, with and without flax plants. Soil was precolonized for 2 weeks by the strains of *Fusarium* initially added to sterilized soil at 1.0×10^3 CFU/g soil (cf. Fig. 1). Population densities in the presence or absence of roots are not significantly different. In each case, absence or presence of roots, columns with common letters are not significantly different by Newmann and Keuls test ($p = 0.05$).

colonized the soil with the lowest level exceeding 4.0×10^5 CFU/g soil. There were clear differences in the ability of the strains to colonize the same sterilized soil. Fo44 followed by Fo47 and Fsp2 were the best colonizers; Fs59, Fs2 and Fo34 also colonized the sterilized soil but reached lower population densities.

Population dynamics of strains of Fusarium *spp. in the presence or absence of flax roots*

Figures 2 and 3 show population densities of strains of *Fusarium* spp. previously

established in sterilized soil, after 21 and 35 days of incubation in the presence or absence of flax roots.

After 21 days (Fig. 2), the population densities were higher in the presence than in the absence of the roots. There was a slight but significant effect of the plant which induced an increase in the population density of the *Fusarium* strains studied (F. test significant at $p = 0.05$), but did not change the ranking of the strains in respect of the population densities reached in sterilized soil.

Moreover, this difference of the population levels in the presence or absence of

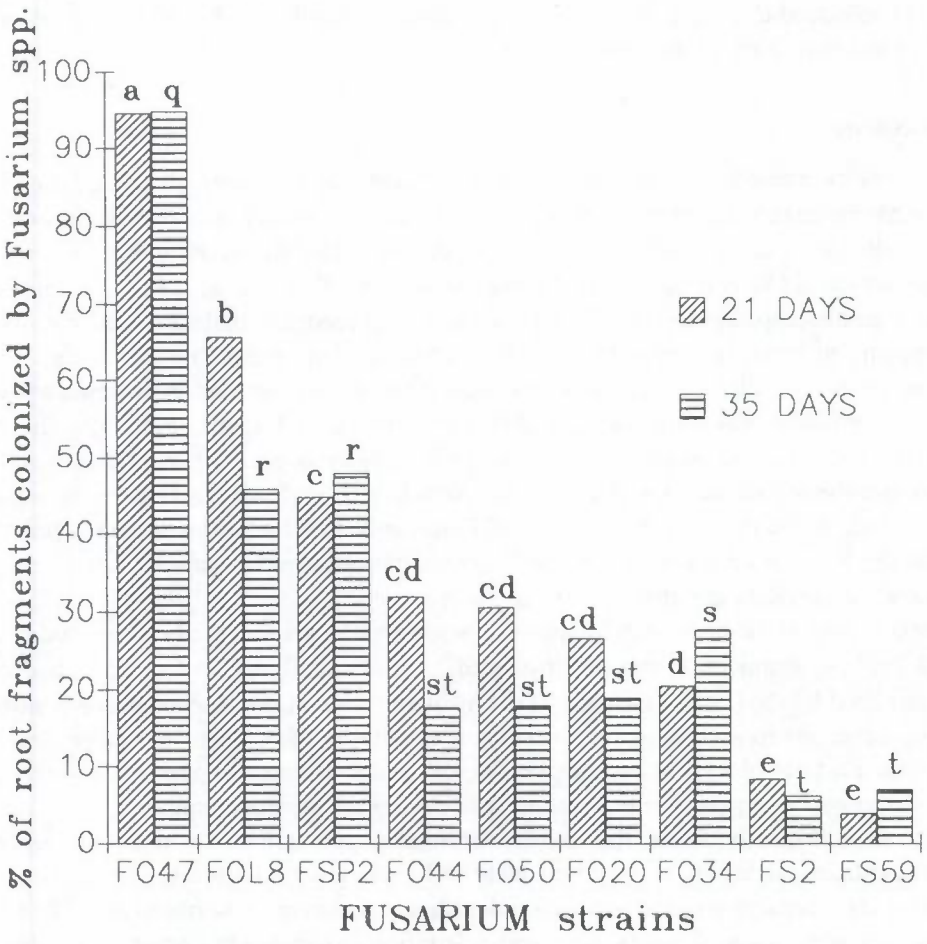

Figure 4. Percent of flax root fragments colonized by nine strains of *Fusarium* after 21 and 35 days. The soils were precolonized by the same strains of *Fusarium* for 2 weeks prior to planting (cf. Fig. 1). For each test period (21 or 35 days) columns with common letters are not significantly different by Newmann and Keuls test ($p = 0.05$).

roots disappeared after 35 days of incubation (Fig. 3). In any case the presence of the plant did not modify the ranking of the strains for their ability to colonize the sterilized soil.

Ability of strains of Fusarium *to colonize roots of flax*

Figure 4 shows the percentages of root pieces of flax colonized by the nonpathogenic strains of *Fusarium* after 21 and 35 days of culture. These strains rapidly colonized the roots and generally the differences between 21 and 35 days were not significant. There was a great diversity in the ability of these nine strains to colonize the roots of flax. Fo47 colonized close to 100% of the root fragments while Fs2 and Fs59 were recovered from less than 10% of the root fragments.

Discussion

Many experiments showed that a massive introduction of a nonpathogenic *Fusarium* into a conducive soil induces suppressiveness to Fusarium-wilts. It was also demonstrated that all strains of nonpathogenic *Fusarium* did not show the same ability to control fusarium-wilts (Alabouvette 1987a; Corman et al., 1986; Paulitz et al., 1987). To improve the screening procedures of efficient strains to be used for biological control, it is necessary to better understand the mode of action of these nonpathogenic strains.

In Dijon, considering that biological control will be first applied in steamed soil or sterile growing substrates for vegetable and horticultural crops under greenhouse conditions, we chose to characterize the specific ability of each strain of *Fusarium* to colonize the soil or the root of a non-host plant in sterilized soil. The aim of this study was to assess the diversity of behaviour of *Fusarium* strains for further studies expecting that the best colonizers would be good competitors in a more complex environment where the nutrients are limited.

According to the population dynamics documented in another study (Couteaudier et al., 1988), a strain of *Fusarium* introduced in a sterilized soil will reach a plateau determined by the limiting capacity of the medium. Thus, in the absence of competition from other microorganisms, the population density at the plateau is related to the specific capacity of a strain to colonize the soil, and differences in population densities reveal disparities in the ability of strains to colonize the sterilized soil.

It is difficult to assess the density of fungal populations in soil. But, under standardized conditions, the dilution plate technique gives accurate results allowing a statistical comparison of the population densities of *Fusarium* in sterilized soil. The first analyses were made 2 weeks after soil infestation, when the *Fusarium* populations reached their plateau, which is independent from the initial inoculum density (Zegerman et al., 1987). The results indicate that the nine strains of *Fusarium* tested had different ability to colonize the sterilized soil. It is possible to delineate groups of *Fusarium* having comparable abilities to colonize the sterilized soil. Fo44, Fo47 and Fsp2 make a group

of good colonizers, their population densities at the plateau are always greater than the population densities of Fs2, Fs59 and Fo34. We may question the validity of such differences in population density, considering that all the strains studied increased their populations by almost 3 log units in sterilized soil. Moreover these results were reproduced in different sterilized soils: the ranking of the strains stays similar and current studies indicate that the best colonizers are the strains that show the best ability to utilize nutrients, especially organic carbon in soil (Couteaudier, Y., 1989). Microscopic observations showed that, under these conditions, the increase of population density was mainly due to the formation of conidia (Couteaudier Y, 1989). Therefore, the capacity of a strain to colonize the soil was related to its capacity to produce conidia. The strains of F. oxysporum studied, mainly produce microconidia while the strains of *F. solani* also produce multicellular macroconidia. The numbers of Colony Forming Units given by the dilution plate technique did not allow to take into account these differences in shape and biomass of the propagules. However it must be stressed that clear differences occured among the strains of *F. oxysporum*, as they existed among the strains of *F. solani*.

The growth of young flax plants did not induce a stable and significant increase in population density of *Fusarium*. Comparisons of populations in soil with or without plants showed a slight but significant increase after 21 days of incubation in the presence of plants, but this rhizospheric effect disappeared after 35 days. The experimental design in which the growing roots pass rapidly through the soil layer may provide an explanation for these results. After 21 and 35 days the root elongation zone where the root exudates are produced is no longer located in the soil but down in the Perlite. It is possible that the stimulation of the population in soil occurred earlier than 21 days, and we measured the end of the phenomenon. Moreover, it is not clearly established that populations of *Fusarium* are stimulated by root exudates. Warren and Kommedahl (1973) reported a higher level of *Fusarium* in rhizosphere soil in comparison with raw soil, but Alabouvette et al., (1984) did not observe any significant difference.

On the other hand, it is clear that the presence of the plant did not affect the ranking of these *Fusarium* strains for their ability to colonize the soil. There is no specific effect of flax root exudates on the population density of the nine nonpathogenic strains.

Isolations made from the 2 cm sections of the main root of flax growing through the infested soil showed a great diversity in the ability of these strains of *Fusarium* to colonize the root. In both experiments, Fo47 was the best colonizer, followed by Fol8, while Fs2 and Fs59 showed a poor ability to colonize the root. We must discuss the validity of the results with regard to the technique used. Root colonization by *Fusarium* was assessed after surface sterilization of the roots. However this technique did not allow to know where the fungus, from which a colony arose, was located. The roots, 21 and 35 days old, were quite mature. Therefore, dead organic material

external to the phellogen could have been colonized by the strains of *Fusarium*. It is quite impossible to clearly distinguish between the saprophytic ability of the strains and their ability to colonize living root tissues. A comparison between the inoculum densities in soil planted with flax and the percentage of root fragments colonized by *Fusarium* spp demonstrates that the ability of these strains to colonize the root is not related to their ability to colonize the soil. Fo44 is the best soil colonizer and a poor root colonizer, while Fo18 is a good root colonizer even when its population density in soil is comparatively low. These results are in agreement with earlier observations showing that root colonization by *Fusarium* spp. was not correlated to the population density of these fungi in both suppressive and conducive soils (Alabouvette et al., 1984b; Schneider, 1984).

Do correlations exist between the ability of a strain to actively colonize the soil or the root cortex and its ability to induce biological control of Fusarium-wilts? This study did not allow us to answer this basic question. The strains Fol8 and Fo47, which are the best in reducing the incidence of *Fusarium* wilt of flax (Corman et al., 1986), also are the best root colonizers. In fact, Fo47 is the nonpathogenic strain we selected for biological control experiments because it performed well in several bioassays (Alabouvette et al., 1987b). However, at the present time it is impossible to attribute the efficiency of Fo47 to its ability to colonize the soil or the root or both together. From a theoretical point of view a strain successful for biological control has to be a good competitor both in soil and at the root surface. We could probably devise simpler methods of screening for efficient strains, if we better understood the mode of action of the nonpathogenic strains of *Fusarium*.

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