Efficacy of Antagonistic *Streptomyces* spp. Isolates and Dose-response Relationship in Biological Control of Fusarium Wilt of Maize

WELLINGTON BRESSAN

Embrapa Milho e Sorgo, Microbiology Department, MG 424, Km 65, C.P. 151, 35701-970, Sete Lagoas, MG, Brazil; e-mail: bressan@cnpms.embrapa.br

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Fusarium moniliforme J.Sheldon (*Giberella fujikuroi*) (Sawada Wollen), commonly infects a wide range of crops and is a major parasite of the Gramineae, particularly in tropical and subtropical regions. On maize (Zea mays L.) the fungus causes seedling blight as well as root, stalk , ear and kernel rot (Galperin et al., 2003). A variable pathogenicity of the fungus has been reported with additional pathogens and stress factor (Velluti et al., 2000).

Biological control of soilborne plant pathogens has shown to be a potential alternative disease management strategy. These agents not only have the potential to protect seeds but they colonize the rhizoplane or rhizosphere when added as seed treatments, and may protect the subterranean portions of growing plants from attack by plant pathogens (Ahmad and Baker, 1987).

Actinomycetes are naturally present in soils and when tested in in vitro condition strains of the genus Streptomyces have shown the potential to produce antibiotics which reduce or inhibit the growth and development of soilborne plant pathogens (Kim et al., 2000; Ouhdouch et al., 2001; Bressan, 2003)

The degree of disease control obtained depends on the density of the biocontrol agent, the density of the of the pathogen, the efficiency of the biocontrol agent in suppressing the pathogen, and the proportion of the pathogen population that is potentially affected by the agent (Montesinos and Bonaterra, 1996; Smith et al. 1997). Differences in the mechanism of action of the biocontrol agents also affect the dose-response relationship of the isolates (Larkin and Fravel, 1999). Biological control agents must be at adequate population levels and be capable of effectively interacting with the pathogens or host to provide acceptable disease control. Knowledge on the relationships between biocontrol agent and pathogen inoculum concentration can determine the population levels of the biocontrol agent that are required to achieve adequate disease control, as well as the pathogen population levels at which the control agent will or will not be effective.

The objective of this work was to evaluate the inoculum concentration relationship between the biocontrol agent and pathogen inoculum on biocontrol of Fusarium wilt in maize by two *Streptomyces* spp. isolates.

The effect of the biocontrol isolates on chlamidospores germination of the pathogen in soil was priminarly assessed according to Larkin and Fravel (1999). The pathogen *Fusarium moniliforme* J. Sheldon (*Giberella fujikuroi*) (Sawada Wollen), was isolated from maize infected seeds of the cultivar BR201, and cultured on potato dextrose agar (PDA) solid media for 10 days at 22 ± 2 °C. Chlamidospores were produced on casein hydrolysate (0.2%) medium. Flasks containing 250 ml of medium was inoculated with agar blocks from 10 day-

old PDA culture plates. Liquid culture were grown for 10 days on a rotary shaker at 135 rpm at 25 °C and propagule suspensions were adjusted to 1 x 10^5 chlamidospores /ml.

The biocontrol *Streptomyces* spp. isolates DAUFPE 11470 and DAUFPE 14632, isolated from maize rizosphere soil in Itapissuma, PE, were provided from the collection isolates of the Department of Antibiotics of the Universidade Federal de Pernambuco, Recife, PE, Brazil. Stock cultures were maintained on agar slant medium ISP2 (Pridham et al., 1956). Biomass inoculum of the *Streptomyces* spp. isolates was produced by liquid fermentation (180 rpm, 48 h at 27 ± 3 °C) in 500 ml Erlenmayer flasks containing 200 ml of medium (Kawamura et al., 1976): soybean meal 20 g/l, glucose 20 g/L, CaCO3 2g/L, NaCl 5 g/L (pH 6.8).

Greenhouse evaluation of dose-response relationship on biological control of Fusarium wilt was made in pots arranged in a completely randomized design experiment with the following treatments: 4 seed antagonists treatments with *Streptomyces* spp. isolates ($1x10^3$, $1x10^4$, $1x10^5$ and 1 $x10^6$ cfu/mL), 4 pathogen soil treatments (inoculation with *Fusarium moniliforme* at $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ chlamidospores/g soil). Control was noninoculated seeds with *Streptomyces* sp. isolates planted in soil inoculated with the pathogen . Five replicates were used for each treatment. Each replicate consisted of 5 plants.

Soil inoculum consisted of propagules of pathogenic *Fusarium oxysporum* on talc powder obtained by the method of Locke and Colhoun (1974) and was incorporated into soil two days before maize seed planting. The final inoculum density in the soil was adjusted to $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ chlamidospores/g soil. Steamed soil was either infested with the pathogen or left untreated.

Maize (*Zea mays*), seeds of the commercial cultivar BR201 were surface disinfected by immersion in a solution of commercial bleach of 5.15% NaClO and 0.5% Tween 20 for 5 minutes and rinsed five times in sterile deionized water under agitation.

Application of *Streptomyces* spp. isolates was made by placing surface disinfected maize seeds in 500 ml beakers with 300 ml of each biomass inoculum, adjusted to $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$ cfu/ml, and shaking continuosly for 24 hours at 28 °C followed by air-drying of the seeds. Non-inoculated seeds were shaken under the same conditions but soaked in sterile deionized water.

A 1:1 mixture of sterilized sandy-loam soil was mixed with talc inoculum of *Fusarium moniliforme* by shaking in a plastic bag. Five seeds for each treatment were sown in plastic pots containing 5 kg of this mixture and watered as required. Disease was monitored for twenty five days after planting and assayed as the total percentage of seedlings showing any symptoms of Fusarium wilt (yellowing, dropping of leaves, vascular discoloration, etc.).

Diseased incidence were confirmed by plating cutting slices from lower stem and roots from diseased seedlings, surface-disinfested in 1% sodium hypochlorite, on Komadas Fusarium – selective medium (Komada, 1975). The experiment was repeated twice.

All data were analysed using SAS (SAS Institute Inc. 1990). Statistical significance was determined at $p<0,05$.

One of the most important strategies used for biocontrol of fungi in crops is to protect the seeds through antagonism microorganisms. Seed treatment with biocontrol agents is one of the most suitable methods for biocontrol of soil borne pathogens in the spermosphere and rhizosphere of field crops (Hebbar et. al., 1992). In this study, two antagonists isolates, previously tested as biocontrol agents against Fusarium wilt, individually demonstrated significant effects in reducing disease incidence at low and hight antagonist and pathogen concentrations. All pathogen and antagonist concentration, significantly ($p<0,05$) affected

development of Fusarium wilt of maize with a significant interaction between pathogen and antagonist concentration (P*A) (Table 1).

Table 1.Factorial analysis of variance table for pathogen and antagonist concentration and interactions on development of Fusarium wilt of maize.

Source of variation	df	MS	
		Antagonist isolate	
		DAUFPE 11470	DAUFPE 14632
Pathogen concentration (P)	3	2409.0*	3266.0*
Antagonist concentration (A)	4	3416.48*	2307.7*
P*A	12	469.77*	381.08*
Error	80	8.61	13.91

[a] df = Degrees of freedom, MS = mean square. Probabilities of less than 0.05 are followed by single asterisks.

This interaction indicates that the level of disease control provided by the antagonists differed among the pathogen concentration. Treatments with each of the two biocontrol isolates (DAUPPE 11470, and DAUFPE 14632), significantly ($p<0.05$) reduced disease compared with the control plants.

When no antagonist was present (pathogen control - C), disease incidence ranged from 19 to 76% with increasing pathogen concentrations. The level of disease control provided by the antagonist isolates differed between the two biocontrol isolates in relation to increasing pathogen concentration. Although, the disease incidence increased with pathogen concentration, for both antagonists isolates and concentrations, it was significantly lower than control, regardless of the pathogen concentration. Differences in efficacy and dose-response relationship between isolates indicate the importance of using different antagonist and pathogen concentrations for the success of the biological control under varying environmetal conditions (Raaijmakers et al. 1995; Larkin and Fravel,1999).

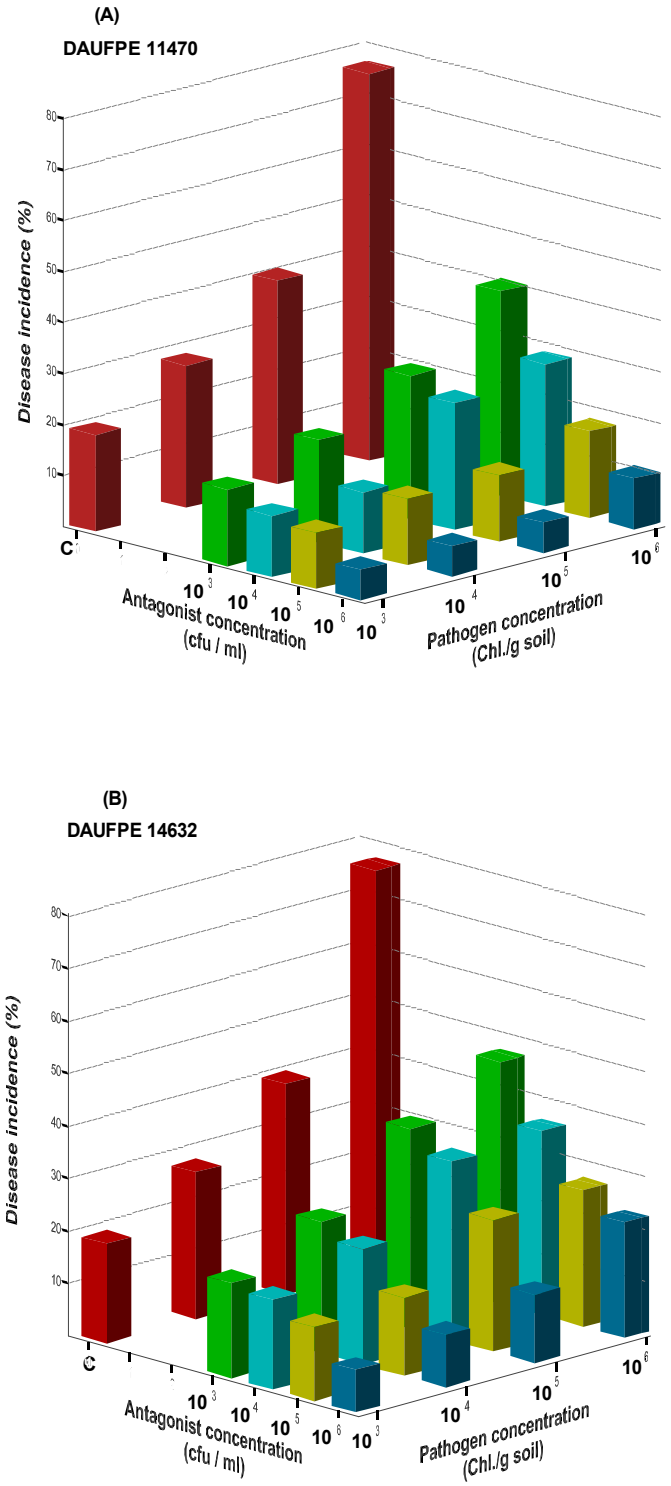

Fig. 1 Effect of varying pathogen and antagonist inoculum concentrations on the development of Fusarium wilt of maize for biocontrol isolates DAUFPE 11470 (A) and DAUFPE 14632 (B).

The hightest disease reduction was obtained when the seeds were inoculated with antagonist isolate DAUFPE 11470, regardless of the pathogen concentration. Considering the pathogen concentration in the soil, the lowest disease reduction, in relation to control plants (soil inoculated with low pathogen concentration and no antagonist inoculation) was 13% and the hightest was 66,2 %, when the the soil and the seeds were inoculated with high concentrations of pathogen and antagonist respectively. However, the lowest Fusarium wilt incidence was observed at the lowest pathogen concentration in the soil and at highest antagonist concentration (Figure 1A). At the lowest pathogen concentration only the seed inoculation with antagonist at $1x10^3$ did not differed significantly ($p<0.05$) from the control plants. Increasing soil pathogen concentration from $1x10^3$ to $1x10^4$ chlamidospores / g soil and antagonist aconcentration from $1x10^4$ to $1x10^5$ (cfu/ml) no significant differences ($p<0.05$) were observed in disease incidence, however the incidence of Fusarium wilt was lower than control in this treatments. At the highest pathogen concentration all seed treatments with antagonists differed significatively ($p<0,05$) from control plants in disease incidence (Fig. 1)

For isolate DAUFPE 14632, in considering the pathogen concentration in the soil, the lowest reduction in Fusarium wilt, in relation to control plants (soil inoculated with low pathogen concentration and no antagonist inoculation) was 10.6%. The hightest reduction, 55%, occured at high antagonist and pathogen concentration when the control consisted of soil inoculation with high pathogen concentration and no antagonist inoculated seeds. However, the lowest disease incidence (8.4 %) was obeserved in the tretament with high antagonist concentration and low pathogen concentration (Fig 1B). At pathogen concentration of $1x10^3$, $1x10^4$ and $1x10^5$ chlamidospores/g soil), no significant ($p<0.05$) reduction in disease incidence, in relation to control plants was observed when increasing antagonist concentration from $1x10^3$ to $1x10^4$ cfu / ml. At the highest pathogen concentration of 10^6 chlamidospores/ g soil no significant differences ($p<0.05$) was observed in disease incidence at antagonist concentration of $1x10^5$ and $1x10^6$.

The results for both isolates showed that the control of Fusarium wilt in maize is affected by the pathogen/antagonist interaction and the dose-response study used in this work was useful for characterizing the differences in the inoculum concentration relationships between the biocontrol isolates and evaluate the effectiveness of the isolates in controlling Fusarium wilt of maize. DAUFPE 11470 was the most desirable biocontrol isolate for Fusarium wilt of the two isolates evaluated and will be used in further studies aiming the improvement of its effectiveness as a biocontrol agent. The results indicated that this study may be applied to biocontrol situations, in which the most important criteria for characterizing disease and biological control are the inoculum concentration of the pathogen and biocontrol agent.

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