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EFFECT OF THE COMBINATION OF SEED PRIMING AND Trichoderma TREATMENT ON INCIDENCE OF DAMPING-OFF AGENTS

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SUMMARY

Cucumber and lettuce seeds were osmoprimed (for 48 and 72 h, respectively) in a mixture of polyethylene glycol solution and Vermiculite. One day before the end of the priming procedure, the process was interrupted in order to dip seeds for 10 min in a spore suspension of Trichoderma koningii. After priming, seeds were planted in sand infested with Pythium sp. Bean seeds were primed in water agar for 24 h, treated with Trichoderma just before priming, and tested in sand infested with Pythium or with R. solani. The percentage of emergence was observed after 10 days of incubation.

Treatment of unprimed seeds with Trichoderma slightly increased stands, relative to seeds unprimed and untreated with Trichoderma. Priming seeds consistently resulted in more rapidly emergence with stands ranging from 62% to 88% (according to the crop-pathogen combinations), as compared to 10% - 26% for the unprimed and Trichoderma-untreated control. Combining Trichoderma treatment and seed priming increased plant stands (emergences ranging from 88% to 100%), relative to untreated primed control, except for the Pythium-lettuce and Rhizoctonia-bean combinations, for which Trichoderma treatment did not provide any additional protection to seed priming in terms of germination percentage.

Primed Trichoderma-treated cucumber and bean seeds were treated with fungicide Sumico in order to kill Trichoderma, and were then planted in sand infested with Pythium . We observed that killing Trichoderma after priming procedure did not modify the protection obtained.

INTRODUCTION

The use of biological control agents is potentially interesting to control soilborne plant diseases. Seed treatments is an attractive delivery system for fungal or bacterial bioprotectants, and in this respect, the use of *Trichoderma* spp. as biological control agents is well documented (13). Biological agents, however, tend to be

somewhat less effective and more variable than chemical pesticides. Thus, developping seed treatment procedures that enhance and stabilize the efficacy of biological control agents of plant pathogens are needed.

One of the most promising physiological methods to enhance the efficacy of biological agents is seed priming (8), in wich controlled hydration initiates the process of germination without radicle emergence(2). Osmopriming (OP) is a presowing treatment where seeds are allowed to imbibe in an aerated osmotic solution, such as polyethylene glygol (PEG) or various salts. The osmotic potential of the solution regulates the amount of water uptake by the seed, enabling the initiation of the germination process(9, 10, 11). Solid matrix priming (SMP) have been developped as an alternative to priming seeds in osmotic solutions. In this procedure, seeds are mixed with a finely ground lignite or coal substance, and sufficient additional water is added to achieve the appropriate moisture potential for priming; the mixture is then incubated for a given time at constant temperature (7).

The purpose of our work was to evaluate the integration of the priming procedures and seed treatments with *Trichoderma*, on the incidence of damping-off fungi, *Pythium* sp. and *Rhizoctonia solani*.

MATERIALS AND METHODS

Seeds and microorganisms

Seeds used in this study were cucumber (*Cucumis sativus*, var. Délicatesse), bean (*Phaseolus vulgaris*, var. Prélude), and lettuce (*Lactuca sativa*, var. Paresseuse).
A strain of *Trichoderma koningii* Rifai obtained from CIMIC (Microbiological Research Center, Andes University of Colombia) was previously selected for its protective properties against *Pythium* sp. and *R. solani* (unpublished results), when incorporated in the soil and as seed coating. Strains of *Pythium* sp. and *R. solani* were isolated in our laboratory from sugar beet with black leg and bean with damping-off symptoms.

Substrate

All experiments were performed in sand sieved through a 4 mm mesh screen and sterilized at 150°C for 6 h. Sowing was performed in plastic pots containing 320 g of sand in which 5 bean seeds, 5 cucumber seeds or 10 lettuce seeds had been planted.

Trichoderma seed treatments and priming procedures

T. koningii was grown on malt extract agar (Difco) at 25°C during 7 days, under a 16 h photoperiod of fluorescent light. Conidia were harvested by scraping the surface of the colonies with a spatula and transferring the slurry to water, to yield

a spore suspension of 10^7 spores per milliliter. Unprimed seeds were dipped for 10 min in this suspension, or in water (control).

Priming of cucumber and lettuce seeds was performed by maintaining them in Petri dishes (185 mm diameter) containing 30 g of Vermiculite moistened with 150 ml of PEG solution (30.2 g/100ml) (15,16), under a layer of Whatman N°1 filter paper. Cucumber and lettuce seeds were then incubated for 48 h and 72 h, respectively, at 25°C under a 16 h photoperiod of fluorescent light. One day before the end of the priming procedure, the treatment was interrupted for 10 min in order to immerse seeds in a spore suspension of *T. koningii* as described above (or in water for control) and was then resumed. Bean seeds were first treated with *T. koningii* as described above, and then primed for 24 h in Petri dishes (85 mm) containing water agar (2%) in a growth chamber.

On priming completion, seeds were rinsed with tap water for 20 sec before being submitted to drying for 48-72 h at 25°C. Seeds were stored then in Petri dishes at 4°C.

In order to kill *Trichoderma* after priming, seeds were dipped for 30 min in 4ppm (a.i) of the fungicide Sumico (25% diethofencarbe and 25% carbendazime)(4), or in water (control), and then dried.

Colonization of bean seeds by *Trichoderma*

Colonization of seeds by *Trichoderma* was evaluated before and after the drying procedure. Three replicates of 2 g of seeds were washed for one minute with 0.05% Tween 20 in sterile distilled water, suspended in 18 ml of sterile distilled water and triturated in a mortar. Serial dilutions were prepared, and 100 µl aliquots of each dilution were plated onto 0.1% sodium desoxycholate PDA, followed by incubation for 5 days at 25°C under a fluorescent light with 16 h photoperiod . Resultant *Trichoderma* counts were expressed as colony-forming units (cfu) per gram of seed.

Growth chamber experiments

Pythium was grown on a nutrient medium containing Vermiculite, V8 juice as additive, and water (20 g/ 24 ml/ 80ml respectively). This medium was autoclaved for 20 min at 120°C, and was inoculated with 4 discs (5mm) of 7 day-old *Pythium* culture grown in Corn meal agar (CM, Difco). *R.solani* was grown on a nutrient medium containing 20 g of Vermiculite and 80 ml of liquid medium YDB (19), autoclaved for 20 min at 120°C, and inoculated with 4 discs (5mm) of 7 day-old *Rhizoctonia* culture grown in MEA. Cultures were incubated for 7 days in the growth chamber as described above.

The sand was inoculated with *Pythium* culture (0.01%, 0.1% or 10% W/W) for cucumber, bean or lettuce experiments respectively, or with *R. solani* (2.5% W/W) for bean experiments.

Primed or unprimed seeds (either treated or not with *T. koningii*) were sown in pots filled with infested or uninfested sand.

Studies were conducted in a growth chamber at 22 ± 1 °C with a photoperiod of 16 h light. Sand moisture holding capacity was maintained at 60%. Stand daily counts started 1 day after planting and continued for 10 days. Counts were expressed as percentages of germination.

There were 5 replicates of each treatment arranged in a randomized complete block design, on growth chamber benches. The study was repeated twice.

RESULTS

Seedling assay with *Pythium*

Emergence of unprimed or *Trichoderma*-untreated seeds in *Pythium*-infested sand were 26%, 16% and 26% in cucumber, bean and lettuce, respectively (Fig 1A, 1B, 1D), and 10% for bean in *R.solani* infested sand (fig 1C).

Trichoderma treatment of unprimed seeds did not modify plant stands for lettuce (in *Pythium*-infested sand), or increased it slightly for all other crop pathogen-combinations, compared to untreated seeds.

In *Pythium*-infested sand, emergence of unprimed seeds compared to primed seeds ranged from 26% to 66% for cucumber, from 16% to 74% for bean, and from 26% to 62% for lettuce. In *R.solani* infested sand, priming also increased bean seeds emergence (from 10% with unprimed seeds to 88%).

Combining seed priming with *Trichoderma* treatment resulted in an increased emergence for cucumber-*Pythium* or bean-*Pythium* combinations. For cucumber, stands ranged from 26% with untreated seeds to 88% with *Trichoderma* treatment of primed seeds. For bean, stands ranged from 16% with untreated seeds to 98% with *Trichoderma* treatment of primed seeds. In lettuce-*Pythium* and bean-*Rhizoctonia* combinations, *Trichoderma* treatment did not provide any additional emergence, but reduced damping-off postemergence symptoms. Conversely, in uninfested sand, unprimed, *Trichoderma*-treated lettuce seeds resulted in more rapid emergence than untreated seeds, and plant height and general robustness were improved (Fig. 2)

Trichoderma population density on seeds

Populations of *Trichoderma* in cucumber or bean seeds was evaluated before and after drying primed *Trichoderma*-treated seeds. In cucumber seeds, population density of *Trichoderma* decreased after drying, from 47×10^4 to 17×10^4 CFU per gram dry weight (64% reduction). Population of *Trichoderma* in bean seeds declined after drying from an initial level of 70×10^3 to 53×10^3 CFU per gram dry weight (24% reduction).

However, killing *Trichoderma* by Sumico treatment after priming, did not modify the protection obtained (Fig. 3)

DISCUSSION

Matrix priming markedly protected seedlings of all crops assayed against *Pythium* and/or *R.solani*. Benefits resulting from the use of matrix priming, could be explained by the release of seed exudates during priming. Osburn *et al.* (15, 16) observed that the rate of exudation by sugar beet seeds during washing and osmopriming, was correlated with the rate

of germination in soil infested with *Pythium*. Elimination of germination inhibitors present in seed coats by washing seems also to be related to damping-off control (14). Other explanation could be that, due to increased rate of emergence and growth, seedling might escape to lethal infection by *Pythium* or *Rhizoctonia*.

The protection of seedlings by *Trichoderma* appears to be dependent on the delivery system. In infested sand, *Trichoderma* seed coating of unprimed seeds increased emergence slightly for bean, significantly for cucumber, but did not provide any protection for lettuce. *Trichoderma* enhanced the positive effect of priming in terms of emergence in cucumber-*Pythium* and bean-*Pythium* seeds. While improving plant vigour in *Pythium*-lettuce and *Rhizoctonia*-bean combinations.

There was a marked increase in the growth rate of *Trichoderma*-treated lettuce seeds in uninfested sand. It was visually apparent, as the plants emerged rapidly and continued to grow through out the period of the experiment. Ahmad and Baker (1), found increased growth responses of plants as a consequence of seed treatment with *Trichoderma* on cucumber, pea, tomato and radish. Windhman *et al.* (20) found that the rate of tomato and tobacco germination was increased, compared to controls where *Trichoderma* spp. was separated from seeds by a cellophane membrane. They concluded that such *Trichoderma* spp. produced a growth-regulating factor that increased the rate of seed germination.

Our data on population dynamics of *Trichoderma* before and after drying primed seeds, showed that the fungus populations declined after drying, while killing *Trichoderma*, by a fungicide treatment applied after priming, did not modify the protection observed. Thus, colonization by, and activity of, *Trichoderma* during the precolonization period, is sufficient to express its protective ability, linked to site occupation(4), removal of exudates (6, 17, 18), production of toxic metabolites (12, 13) and/or hydrolytic enzymes (3, 5). The fact that killing *Trichoderma* after priming did not affect the protective effect, excludes mycoparasitism as a mode of action.

One of the limiting factors in the development of biological control products has been their low reliability, variable efficacy and narrow spectrum of activity.

Priming seeds in the presence of *Trichoderma* spores seems an attractive method of protection against damping-off agents; it promoted reproducible effects in terms of high emergence percentage, improve the vigour of the plants and reduced post-emergence symptoms, with several crop-pathogen combinations. From a practical point of view, priming avoids the problem of survival of the biocontrol agent in treated seeds.

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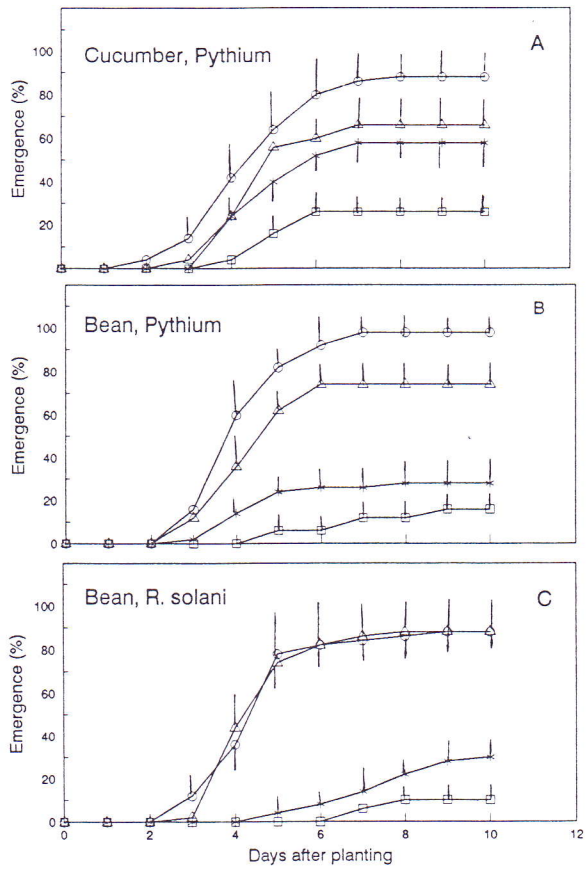

Fig 1. Emergence percentage of (A) cucumber, (B,C) or bean grown from unprimed□—□, primed△—△, unprimed+*Trichoderma*✳—✳, and primed+*Trichoderma* ○—○ seeds. Seeds were planted in sand artificially infested with *Pythium*, (cucumber, bean and lettuce) or with *R. solani* (bean seeds). The bars represent the standard deviation about each point.

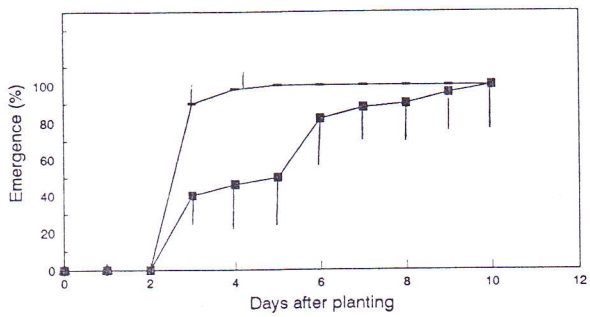

Fig 2. Emergence percentage of unprimed lettuce seeds in uninfested sand, untreated control or treated with *Trichoderma*

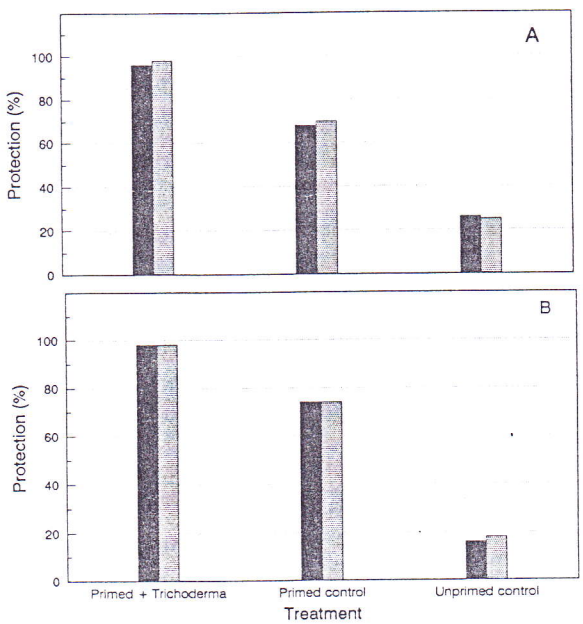

Fig 3. Effect of killing *Trichoderma* after priming on the protection against *Pythium* (A) cucumber seeds (B) bean seeds . Seeds were treated with water (*Trichoderma* still alive) or with Sumico (*Trichoderma* is killed).

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