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(54) Titre : LUTTE CONTRE LES AGENTS PATHOGENES DE PLANTES A L'AIDE DE COMBINAISONS
BACTERIENNES ET FONGIQUES ANTAGONISTES

(54) Title: CONTROLLING PLANT PATHOGENS WITH FUNGAL/BACTERIAL ANTAGONIST COMBINATIONS
COMPRISING TRICHODERMA VIRENS AND BACILLUS AMYLOLIQUEFACIENS

(57) **Abrégé/Abstract:**

Fungal/bacterial antagonist combinations, a seed coated with one of the combinations and a plant protected from plant pathogens by one of the combinations. The invention is also a fungal/bacterial antagonist combination and its use for controlling plant pathogens as a biocontrol agent, bio-pesticide or bio-fungicide. The invention also finds utility as a fungal/bacterial antagonist combination applied to the seed, stalk or leaf that results in an increase in plant yield.



ABSTRACT OF THE DISCLOSURE

Fungal/bacterial antagonist combinations, a seed coated with one of the combinations and a plant protected from plant pathogens by one of the combinations. The invention is also a
5 fungal/bacterial antagonist combination and its use for controlling plant pathogens as a biocontrol agent, bio-pesticide or bio-fungicide. The invention also finds utility as a fungal/bacterial antagonist combination applied to the seed, stalk or leaf that results in an increase in plant yield.

CONTROLLING PLANT PATHOGENS WITH FUNGAL/BACTERIAL ANTAGONIST
COMBINATIONS COMPRISING *TRICHODERMA VIRENS* AND *BACILLUS*
AMYLOLIQUEFACIENS

5 Thomas D. Johnson

FIELD OF THE INVENTION

10 This invention relates to fungal/bacterial antagonist combinations, a seed coated with said combinations and a plant protected from plant pathogens by said combinations. In particular, the invention relates to fungal/bacterial antagonist combinations and their use for controlling plant pathogens.

BACKGROUND OF THE INVENTION

15 Early and late season stalk and root rot are major causes of crop loss. A variety of plants are affected, including tomatoes, peppers, turf grass, soybeans, sunflower, wheat and corn. The pathogens that cause these symptoms include fungi of the genera *Fusarium*, *Phythium*, *Phytophthora* and *Penicillium*.

20 One approach to solving the problem of early season damping off of plants is treatment of seeds with fungicides, such as captan, metalaxyl and Maxim. Although these chemicals enhance seed germination and seedling stand by inhibiting the pathogenic ability of *Phythium* spp. (active

in cool, wet soils), they have no activity against the pathogenic fungi that are responsible for late season root and stalk rot.

Fusarium and Penicillium are the pathogens responsible for late season root and stalk rot.

5 These pathogens prefer the warm, dry conditions that occur late in the growing season. There is no chemical or biological fungicide available that addresses the problem of late season root and stalk rot in corn. Currently, the only way to deal with this problem is to periodically rotate to a non-susceptible crop to reduce pathogen numbers. Corn growers can also select hybrids that have better "standability," but such hybrids usually have lower yields. Unfortunately, the corn
10 varieties with the highest yields are usually those most susceptible to late season root and stalk rot.

Trichoderma is a genus of fungi that contains about 20 species. Synonyms for the genus name include Aleurisma and Sporoderma. *Trichoderma virens*, which is also called *Gliocladium*
15 *virens*, is a member of the genus. The natural habitats of these fungi include soil and plant material. A member of the genus, *Trichoderma harzianum* KRL-AG2 (ATCC 20847) also known as strain T-22, is used as a biocontrol agent that is applied as a seed or soil treatment or on cuttings and transplants. Strains of the species, *Trichoderma virens*, have also been used for control of damping off diseases in plants. For example, *Gliocladium virens* GL-21 is sold under
20 the trade name SoilGard® (formerly GlioGard).

Bacillus is a genus of rod-shaped, gram-positive, aerobic or (under some conditions) anaerobic bacteria. Bacillus species are widely found in soil and water and some have been used to control plant diseases, including root rot. *Bacillus amyloliquefaciens* is a spore-forming member of the genus. *Bacillus amyloliquefaciens* L.L. Campbell strain F (ATCC 23350) is the type strain for the species. Other known and commercially available *Bacillus amyloliquefaciens* strains include those having the following ATCC accession numbers: 23842, 23843, 23844 and 23845 (Int. J. Syst. Bacteriol. 37:69-71, 1987; J. Bacteriol. 94:1124-1130, 1967).

Bacillus lentimorbus is another spore-forming member of the genus. *Bacillus lentimorbus* Dutky 1940 (ATCC 14707) is the type strain for the species (Skerman, V.B.D., McGowan, V., and Sneath, P.H.A., Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* 30: 225-420, 1980). Some researchers consider *Bacillus lentimorbus* to be a variety of *Bacillus popilliae*. *Bacillus lentimorbus* and *Bacillus popilliae* have recently been reclassified as *Paenibacillus lentimorbus* and *Paenibacillus popilliae* (Pettersson, B., Rippere, K.E., Yousten, A.A. and Priest, F.G., Transfer of *Bacillus lentimorbus* and *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov., *Int. J. Syst. Bacteriol.* 49: 531-540, 1999). *Bacillus lentimorbus* and *Bacillus popilliae* are the causative agents of milky disease in Japanese beetles and related scarab larvae and "milky spore" powders are sold under the trade names, "Doom," "Milky Spore," "Japidemic" "Grub Killer" and "Grub Attack," for biocontrol of these insects.

Background art biocontrol products have comprised the bacterium *Burkholderia cepacia*, which is also known as *Pseudomonas cepacia*. This bacterium has been implicated as a

human pathogen. Furthermore, it has little or no shelf life unless refrigerated at 4 degrees Centigrade at a minimum of 20 percent moisture.

The background art is characterized by U.S. Patent Nos. 4,476,881; 4,489,161; 4,642,131;
 5 4,668,512; 4,678,669; 4,713,342; 4,724,147; 4,748,021; 4,818,530; 4,828,600; 4,877,738;
 4,915,944; 4,952,229; 5,047,239; 5,049,379; 5,071,462; 5,068,105; 5,084,272; 5,194,258;
 5,238,690; 5,260,213; 5,266,316; 5,273,749; 5,300,127; 5,344,647; 5,401,655; 5,422,107;
 5,455,028; 5,409,509; 5,552,138; 5,589,381; 5,614,188; 5,628,144; 5,632,987; 5,645,831;
 5,665,354; 5,667,779; 5,695,982; 5,702,701; 5,753,222; 5,852,054; 5,869,042; 5,882,641;
 10 5,882,915; 5,906,818; 5,916,029; 5,919,447; 5,922,603; 5,972,689; 5,974,734; 5,994,117;
 5,998,196; 6,015,553; 6,017,525; 6,030,610; 6,033,659; 6,060,051; and 6,103,228.

Gravely et al. in U.S. Patent No. 4,476,881 disclose a mixed complementary culture of
 bacteria and fungi that is used to degrade pectin and cellulose components of tobacco materials.
 15 The invention is limited in that it teaches use of different microorganisms for a different purpose.

Papavizas in U.S. Patent No. 4,489,161 discloses a strain of the fungus *Trichoderma*
viride that is an effective biocontrol agent for *Fusarium* wilt of chrysanthemum. The invention is
 limited in that it teaches use of a different microorganism.

20 Hoitinik in U.S. Patent No. 4,642,131 discloses a process for production of a disease-
 suppressive compost and a microorganism culture for use therein. The invention is limited in
 that it teaches use of different microorganisms for a different purpose.

Lewis et al. in U.S. Patent No. 4,668,512 disclose a method for preparing pellets containing living biocontrol fungi and nutrients. The invention is limited in that it teaches a process that involves use of different microorganisms.

5

Ricard in U.S. Patent No. 4,678,669 discloses a method of using immunizing commensals to control soil-borne pathogens. The invention is limited in that it teaches use of different microorganisms.

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Chet et al. in U.S. Patent No. 4,713,342 disclose a novel isolate of Trichoderma and its use. The invention is limited in that it teaches use of a different microorganism.

15

Marois et al in U.S. Patent No. 4,724,147 disclose a method for preparing pellets containing living biocontrol fungi. The invention is limited in that it teaches a process that involves use of different microorganisms.

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Chet et al. in U.S. Patent No. 4,748,021 disclose antifungal compositions containing Trichoderma active against Fusarium. The invention is limited in that it teaches use of a different microorganism.

Marois et al. in U.S. Patent No. 4,818,530 disclose a method for preparing pellets containing living biocontrol fungi. The invention is limited in that it teaches a process that involves use of different microorganisms.

McCabe et al. in U.S. Patent No. 4,828,600 disclose a biological inoculant for corn. The invention is limited in that it teaches use of different microorganisms.

5 Handelsman et al. in U.S. Patent No. 4,877,738 disclose a new microorganism culture and a method for biological control of damping off and root rot. The invention is limited in that it teaches a process that involves use of a different microorganism, *Bacillus cereus*.

10 Chet et al. in U.S. Patent No. 4,915,944 disclose a novel isolate of *Trichoderma* and fungicidal compositions containing it. The invention is limited in that it teaches use of a different microorganism.

Muir in U.S. Patent No. 4,952,229 discloses a soil and foliar supplement. The invention is limited in that it teaches use of different microorganisms.

15

Pusey in U.S. Patent No. 5,047,239 discloses a biological control agent for fruit rot. The invention is limited in that it teaches use of a different microorganism for a different purpose.

20 Handelsman et al. in U.S. Patent No. 5,049,379 disclose a fungicidal toxin and a method and an inoculum for controlling root rot and damping off. The invention is limited in that it teaches use of a different microorganism, *Bacillus cereus*.

Kimura in U.S. Patent No. 5,071,462 discloses a method and apparatus for producing an organic fertilizer. The invention is limited in that it teaches use of different microorganisms for a different purpose.

5 Lewis et al. in U.S. Patent No. 5,068,105 disclose a fungal formulation for biocontrol of soil-borne plant pathogens. The invention is limited in that it teaches use of different microorganisms.

10 Speakman et al. in U.S. Patent No. 5,084,272 disclose a Trichoderma fungus and a fungicide that contains it. The invention is limited in that it teaches use of a different microorganism.

15 Pauu et al. in U.S. Patent No. 5,194,258 disclose a method for producing enhanced biocontrol agents. The invention is limited in that it teaches use of different microorganisms.

 Elad et al. in U.S. Patent No. 5,238,690 disclose a novel Trichoderma culture and biological compositions containing it. The invention is limited in that it teaches use of different microorganisms for a different purpose.

20 Harman et al. in U.S. Patent No. 5,260,213 disclose fused biocontrol agents. The invention is limited in that it teaches use of different microorganisms.

Elad et al. in U.S. Patent No. 5,266,316 disclose a novel isolate of *Trichoderma harzianum* and fungicidal compositions containing it. The invention is limited in that it teaches use of different microorganisms for a different purpose.

5 Bok et al. in U.S. Patent No. 5,273,749 disclose a process for preparing coated microbial pesticides and the products of the process. The invention is limited in that it teaches use of different microorganisms.

10 Williams in U.S. Patent No. 5,300,127 discloses seed coatings. The invention is limited in that it teaches use of different microorganisms.

Rossall in U.S. Patent No. 5,344,647 discloses a *Bacillus* strain that has antimicrobial activity. The invention is limited in that it teaches use of different microorganisms.

15 Kijima et al. in U.S. Patent No. 5,401,655 disclose a process for biologically preventing plant diseases. The invention is limited in that it teaches use of different microorganisms for a different purpose.

20 Kubota in U.S. Patent No. 5,422,107 discloses a novel fungus and a fungicide containing it. The invention is limited in that it teaches use of a different microorganism.

O'Donnell in U.S. Patent No. 5,455,028 discloses a method of inhibiting fungi. The invention is limited in that it teaches use of a different microorganism.

Burth et al. in U.S. Patent No. 5,409,509 disclose a seed treatment. The invention is limited in that it teaches use of different microorganisms.

Shanmuganathan in U.S. Patent No. 5,525,132 discloses “compositions for the treatment/prevention of microbial diseases of fruit comprising as effective amount of at least one yeast strain . . .” (Abstract). In Example 2, strains are tested separately and are not combined. The disclosed strain D9 was effective and *Trichoderma viridie* was not. Neither was *Bacillus subtilis* effective. The reference teaches that “it is very unpredictable whether a particular yeast species would be effective in the treatment/prevention of microbial disease in fruit” (col. 2, lines 26-28).

Handelsman et al. in U.S. Patent No. 5,552,138 disclose a novel strain of *Bacillus cerus* and a method of protecting plants with the strain. The invention is limited in that it teaches use of a different microorganism.

Neyra et al. in U.S. Patent No. 5,589,381 disclose a novel strain of *Bacillus licheniformis* that produces an antifungal agent and a use for the strain. The invention is limited in that it teaches use of a different microorganism.

Urano et al. in U.S. Patent No. 5,614,188 disclose an anti-Fusarium composition containing strains of *Bacillus* sp. The invention is limited in that it teaches use of different microorganisms.

Eastin in U.S. Patent No. 5,628,144 discloses solid matrix priming of seeds. The invention is limited in that it teaches use of different microorganisms.

Payne et al. in U.S. Patent No. 5,632,987 disclose a *Bacillus thuringiensis* toxin that is active against corn rootworm larvae. The invention is limited in that it teaches use of a different microorganism for a different purpose.

Chilcott et al. in U.S. Patent No. 5,645,831 disclose a *Bacillus thuringiensis* strain and metabolite that are active against corn rootworm. The invention is limited in that it teaches use of a different microorganism for a different purpose.

Neyra et al. in U.S. Patent No. 5,665,354 disclose a novel strain of *Bacillus licheniformis* that produces an antifungal agent and a use for the strain. The invention is limited in that it teaches use of a different microorganism.

Kubo in U.S. Patent No. 5,667,779 discloses a fungi-inhibiting composition comprising *Bacillus subtilis*. The invention is limited in that it teaches use of a different microorganism.

Handelsman et al. in U.S. Patent No. 5,695,982 discloses canavanine-resistant strains of *Bacillus cereus*. The invention is limited in that it teaches use of different microorganisms.

Neyra et al. in U.S. Patent No. 5,697,186 disclose "flocculated bacterial cells" for use "as high-density crop inoculants" (Abstract). The reference is limited to the flocculation of one or

more species of bacteria. Combination of bacteria with another type of microorganism is not taught by the reference. Nor, does it teach the combination of more than one bacterium will produce a greater response, only that the flocculation will allow the application of more than one bacterium for whatever reason.

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O'Donnell in U.S. Patent No. 5,702,701 discloses a process for treatment of soil and plants with a composition containing *Bacillus laterosporus*. The invention is limited in that it teaches use of a different microorganism.

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Marrone et al. in U.S. Patent No. 5,753,222 disclose an antibiotic-producing strain of *Bacillus* and methods of controlling plant diseases with it. The invention is limited in that it teaches use of a different microorganism.

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Handelsman et al. in U.S. Patent No. 5,852,054 disclose fungicidal toxins from a biocontrol bacterium. The invention is limited in that it teaches use of a different microorganism.

20

Marrone et al. in U.S. Patent No. 5,869,042 disclose methods for controlling above-ground plant diseases. The invention is limited in that it teaches use of different microorganisms for a different purpose.

Shetty in U.S. Patent No. 5,882,641 discloses fruit pomice compositions and their uses. The invention is limited in that it teaches use of different microorganisms.

Howell in U.S. Patent No. 5,882,915 discloses viridiol-deficient strains of *Trichoderma virens* and process for making and using biocontrol agents that contain them. The invention is limited in that it teaches use of different microorganisms.

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Heins et al. in U.S. Patent No. 5,906,818 disclose a *Bacillus mycoides* strain for controlling corn rootworm. The invention is limited in that it teaches use of a different microorganism for a different purpose.

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Smith et al. in U.S. Patent No. 5,916,029 disclose a process for producing seeds coated with a microbial composition. The invention is limited in that it teaches use of different microorganisms.

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Marrone et al. in U.S. Patent No. 5,919,447 disclose a strain of *Bacillus* for controlling plant disease. The invention is limited in that it teaches use of a different microorganism.

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Herrera-Estrella et al. in U.S. Patent No. 5,922,603 disclose a method for obtaining strains of *Trichoderma* sp. The invention is limited in that it teaches use of a different microorganism.

Cook et al. in U.S. Patent No. 5,972,689 disclose methods and compositions for control of root diseases. The invention is limited in that it teaches use of different microorganisms.

Eastin in U.S. Patent No. 5,974,734 discloses solid-matrix priming of seeds with microorganisms. The invention is limited in that it teaches use of different microorganisms.

Handelsman et al. in U.S. Patent No. 5,998,196 disclose a method for suppressing disease using a novel *Bacillus cereus* strain. The invention is limited in that it teaches use of a different microorganism.

Germida et al. in U.S. Patent No. 6,015,553 disclose a *Bacillus subtilis* strain for controlling pests. The invention is limited in that it teaches use of a different microorganism for a different purpose.

Logan et al. in U.S. Patent No. 6,017,525 discloses a method of poultry house litter treatment. The invention is limited in that it teaches use of a different microorganism for a different purpose.

Handelsman et al. in U.S. Patent No. 6,030,610 disclose a novel strain of *Bacillus cereus*. The invention is limited in that it teaches use of a different microorganism.

Handelsman et al. in U.S. Patent No. 6,033,659 disclose a novel strain of *Bacillus cereus*. The invention is limited in that it teaches use of a different microorganism.

Heins et al. in U.S. Patent No. 6,103,228 discloses compositions and methods for controlling plant pests and a novel strain of *Bacillus subtilis*. The invention is limited in that it teaches use of a different microorganism.

5 Ocamb et al. in U.S. Patent No. 6,133,196 disclose contacting conifer seeds with a culture of certain bacteria, drying the seeds, planting the seeds and then treating the plant growth medium with a certain ectomycorrhizal fungus (*Hebeloma* spp.). The reference does not teach treating seeds with a composition comprising a fungus and a bacterium.

10 Branly et al. in U.S. Patent No. 6,232,270 discloses agricultural compositions containing bacteria. The invention is limited in that it teaches use of different microorganisms and a different process for their use. While the patent suggests that one bacterium that is useful within the disclosed invention is *Bacillus amyloliquefaciens*, it teaches that spores, cultures or suspensions of the bacterium must be combined with an agriculturally effective ingredient of a
15 plant growth stunting agent or herbicide.

 Moesinger in U.S. Patent No. 6,326,016 discloses plant immunization compositions. The invention is limited in that it teaches use of different microorganisms and a different process for their use. While the patent suggests that one bacterium that can be used according to the
20 disclosed invention is *Bacillus amyloliquefaciens*, and that one fungus that can be used according to the invention is *Trichoderma* spp., it teaches production of an extract by means of biotechnological fermentation process performed by only one microorganism. Furthermore, the

patent teaches that high-temperature autoclaving (e.g., one hour at +120 degrees C) and (optionally) filtering are further steps in preparation of the extract.

Background art is also disclosed in the non-patent literature. The StratSoy Research Database posted on the Web (apparently in 1996) a description of a research project entitled Interaction of Soybean Roots with Soilborne Pathogens and Nonpathogens. The project studied the use of *Bacillus amyloliquefaciens* B94 as a biocontrol agent for plant diseases. The invention is limited in that it teaches use of a single different microorganism.

An article entitled Seed's Coat of Many Microbes Wards Off Rot in Science Update section of the April, 1997, issue of Agricultural Research, disclosed the mixing of helpful fungi and bacteria to weaken or kill fungal pathogens that attack young corn sprouts. The applicant believes that the combination used was *T. virens* and *Pseudomonas cepacia*. This invention is limited for two reasons: (1) *P. cepacia* is implicated as causative in deaths in cystic fibrosis patients and, therefore, EPA registration would probably not be possible, and (2) *P. cepacia* is not a spore-forming bacterium and, therefore, the moisture content of the combination must be maintained at greater than about 17 percent. *T. virens* can be stored and applied at low moisture levels and does not remain in the spore state at the higher moisture levels, making its combination with *P. cepacia* impractical.

In an article authored by L.E. Datnoff et al. entitled Effect of Bacterial and Fungal Microorganisms to Colonize Tomato Roots, Improve Transplant Growth and Control Fusarium Crown and Root Rot that was posted on the Web in 2000 by the University of Florida Southwest

Florida Research and Education Center, the authors discuss using a combination of an unidentified bacterial strain and *Gliocladium virens* to control Fusarium root rot. The invention is limited in that it teaches use of different microorganisms.

5 The U.S. Environmental Protection Agency Office of Pesticide Programs posted a Biopesticide Fact Sheet on *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 (006480) in May, 2000. The fact sheet noted that the strain was approved for use as a growth enhancer and disease suppressor. The invention is limited in that it does not teach the use of a fungal/bacterial combination.

10 No single reference and no combination of the references teach the invention disclosed herein. The background art does not teach combinations of microorganisms disclosed herein, combinations that provide a surprising consistency of performance in plant disease control.

15 **BRIEF SUMMARY OF THE INVENTION**

 A purpose of the invention is to control the plant pathogens that cause early and late season root and stalk rot. Another purpose is to provide for season-long protection for plants from the pathogens that cause early and late season root and stalk rot. Another purpose is to provide consistent disease control for plants. Yet another purpose is to increase the yield of plants and plant seed production.

20

One advantage of the invention is that root and stalk rot can be controlled with a composition that is not toxic to humans. Another advantage of the invention is that root and stalk rot can be controlled more economically than with chemical fungicides. Yet another advantage of the invention is that it provides a biocontrol agent or bio-pesticide with extended shelf life. Thus, a seed can be treated with the biocontrol agent and stored for a period of months and still host a viable biocontrol agent that will colonize the root when the seed is placed in the ground, germinates and grows. Furthermore, the disclosed biocontrol agent is competitive with natural soil microbes that occur in the rhizosphere while providing pathogen protection for the plant. A further advantage of the invention is that the combination of a fungal/bacterial antagonist is more effective in controlling fungal pathogens in the plant rhizosphere than either a fungal antagonist or a bacterial antagonist alone. Thus, the invention provides an easy-to-use, effective means of controlling plant pathogens that have been only been controllable by rotation management. A further advantage of the invention is that its use produces more consistent results than the use of either a fungal antagonist or a bacterial antagonist alone, as shown by the Working Examples presented herein. In fact, use of the antagonist combinations disclosed herein is shown to be functional when use of its individual constituent antagonists is not.

The compositions disclosed herein may be integrated into Integrated Pest Management (IPM) programs, the inventive compositions may be used in combination with other management systems. As an alternative to synthetic agents, biocontrol agents (bio-pesticides) offer the advantage of containing naturally derived constituents that are safe to both humans and the environment. Specifically, bio-pesticides offer such advantages as being inherently less toxic than conventional pesticides, generally affecting only the target pest and closely related

organisms, and are often effective in very small quantities. For these reasons, bio-pesticides often decompose quickly and, therefore, are ideal for use as a component of Integrated Pest Management (IPM) programs.

5 The invention is an inoculum, a seed coated with the inoculum, a plant protected with the inoculum, a method of producing the inoculum and a method of protecting a seed or a plant with the inoculum. A further embodiment of the inoculum comprises a combination of a fungus and a bacterium. Preferably, the fungus is a species of *Trichoderma* and the bacterium is a species of *Bacillus*, preferably a spore-forming strain of *Bacillus*. More preferably, the fungus is
10 *Trichoderma virens* and the bacterium is *Bacillus amyloliquefaciens*, although other combinations are also envisioned. Even more preferably, the fungus is *Trichoderma virens* G1-3 (ATCC 58678) and the bacterium is *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390).

15 Further embodiments of the invention comprise combining of a *Trichoderma virens* fungus and a *Bacillus amyloliquefaciens* bacterium and placing this combination on a seed or in the vicinity of the seed or seedling. A routineer would understand that other correct names/designations for the specific isolates/strains involved are: *T. virens* isolate G1-3 and *B. subtilis* var. *amyloliquefaciens* strain TJ 1000. A routineer would also understand that the names
20 *Trichoderma virens* and *Gliocladium virens* are synonymous. To confirm that *Trichoderma virens* G1-3 is a true *Gliocladium*, the ATCC listing of this organism under ATCC Accession No. 58678 confirms its prior classification as a *Gliocladium virens*. The ATCC site lists the claimed isolate (58678) as G1-3.

In a further embodiment, the inoculum is produced by adding an essentially pure culture, a substantially pure culture, an axenic culture or a biologically pure culture of *Trichoderma virens* G1-3 to a bioreactor containing molasses-yeast extract growth medium using a standard inoculation technique. The medium is agitated and aerated and its temperature is maintained at about 28 degrees Centigrade. After the *Trichoderma virens* G1-3 is grown in the medium for about eight hours, an essentially pure culture, a substantially pure culture, an axenic culture or a biologically pure culture of *Bacillus amyloliquefaciens* TJ1000 or 1BE is added to the medium using a standard inoculation technique. The combined, competitive culture is grown under the aforementioned conditions and produces maximum cell and spore counts in approximately seven days. The combined culture is then used as an inoculum and is applied each seed at a rate of no less than about 1,000 spore counts per seed.

In a further embodiment, a solution containing an essentially pure culture, a substantially pure culture, an axenic culture or a biologically pure culture of the fungal antagonist *Trichoderma virens* G1-3 is combined with a solution containing an essentially pure culture, a substantially pure culture, an axenic culture or a biologically pure culture of *Bacillus amyloliquefaciens* TJ1000 or 1BE in a 50/50 mixture by volume and is applied to a seed at a rate of no less than about 10,000 spore counts per seed.

In broad terms, a further embodiment of the invention is an agricultural inoculum suitable for inoculating plant seeds comprising a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof, a bacterial antagonist selected

from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof, and a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal. Suitable carriers include wettable clay based powders, dextrose granules or powders, sucrose granules or powders and maltose-dextrose granules or powders.

5

A further embodiment of the invention is a composition of matter comprising a plant seed inoculated with a combination comprising a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof and a bacterial antagonist selected from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof, wherein said combination suppresses growth of plant pathogenic fungi.

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Yet a further embodiment of the invention is a seed or plant inoculated with a combination comprising a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof and a bacterial antagonist selected from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof, wherein the combination suppresses growth of plant pathogenic fungi.

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In broad terms, a further embodiment of the invention is a method of protecting a plant from disease caused by a plant pathogenic fungus comprising inoculating seeds from said plant with a combination comprising a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof and a bacterial antagonist selected from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof, wherein said combination suppresses growth of plant pathogenic fungi.

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A further embodiment of the invention is a method of protecting a seed or a plant from disease caused by a plant pathogenic fungus comprising inoculating seeds from said plant with a composition comprising a spore-forming fungal antagonist and a spore-forming bacterial antagonist. Preferably, the spore-forming bacterial antagonist is selected from the group *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof.

A further embodiment of the invention is a method of protecting a seed or a plant from disease caused by a plant pathogenic fungus comprising inoculating seeds from said plant with a composition comprising a fungal antagonist and a bacterial antagonist, wherein said combination suppresses growth of plant pathogenic fungi. A further embodiment is capable of control of the plant pathogen fungi *Fusarium*, *Phythium*, *Phytophthora* and *Penicillium*.

A further embodiment of the invention is a method of protecting a plant from disease caused by a plant pathogenic fungus comprising inoculating seeds from said plant with a composition comprising a fungal antagonist and a bacterial antagonist selected from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof, wherein said combination suppresses growth of plant pathogenic fungi.

Yet A further embodiment of the invention is a method for biologically controlling or inhibiting stalk rot or root rot comprising coating seeds with an effective amount of a composition comprising *Trichoderma virens* G1-3 (ATCC 58678) and *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390).

A further embodiment of the invention is process for making a composition comprising introducing an essentially pure culture of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) to a growth medium about eight hours after an essentially pure culture of

5 *Trichoderma virens* G1-3 (ATCC 58678) is introduced to the growth medium and growing the culture as a competitive culture.

A further embodiment of the invention is a process comprising making a composition by combining an essentially pure culture of *Trichoderma virens* G1-3 (ATCC 58678) with an

10 essentially pure culture of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) in a 50:50 mixture and applying said composition to a seed at a rate of at least 100,000 spores per seed.

In one embodiment of the invention disclosed herein, the spore count applied per seed

15 ranges from about 1,000 to about 1,000,000, regardless of seed size. In another embodiment of the invention, the spore count per seed is from about 1,000 to about 10,000. In a further embodiment of the invention, the spore count per seed is from about 10,000 to about 100,000. In a yet further embodiment of the invention, the spore count per seed is from about 100,000 to about 1,000,000. In a yet another embodiment of the invention, the spore count per seed is from

20 about 1,000,000 to about 2,000,000.

A further embodiment of the invention is a method for protecting plants in a growing medium from damping off and root rot fungal plant disease comprising placing in the growing

medium in the immediate vicinity of the plant to be protected an effective quantity of one of the fungal/ bacterial combinations disclosed herein.

Yet a further embodiment of the invention is a method for protecting plants from fungal
5 plant disease comprising adding one of the fungal/bacterial combinations disclosed herein in an effective quantity to a substrate such as pelletized calcium sulfate or pelletized lime and placing the pellet in the immediate vicinity of the plant to be protected. The pellet may or may not contain other nutrients.

10 A further embodiment of the invention is a method for protecting plants from fungal plant disease comprising adding one of the fungal/bacterial combinations disclosed herein in an effective quantity to a liquid solution such as water and applying the liquid solution in the immediate vicinity of the plant to be protected. The liquid may or may not contain additional nutrients and may include a chemical fungicide applied to the seed such as, for example, Maxim
15 or captan. The disclosed combination may also be added to a plant nutrient (nitrogen-phosphorus-potassium (NPK)) plus plant micro-nutrient solution that is compatible with the combination and applied as an in-furrow treatment.

A further embodiment of the invention is a method for biologically controlling a plant
20 disease caused by a plant-colonizing fungus, the method comprising inoculating a seed of the plant with an effective amount of a microbial inoculant comprising a combination of microorganisms having all of the identifying characteristics of *Trichoderma virens* G1-3 and *Bacillus amyloliquefaciens* TJ1000 or 1BE, said inoculation resulting in the control of said plant

disease. The invention is also a method according to the above further embodiment wherein said inoculation results in the control of more than one plant disease.

Yet a further embodiment of the invention involves combining of a spore forming fungal strain and a spore forming bacterial strain to enhance ease of use and longevity of shelf life both as a stored product and when applied to a seed. In a further embodiment, the invention involves applying the disclosed *Trichoderma* microorganism and the *Bacillus* microorganism to a wettable powder, in which form it is applied.

A further embodiment of the invention is composition of matter made by combining: a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof; a bacterial antagonist selected from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof; and a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal.

A further embodiment of the invention is an antagonist for controlling plant pathogens made by combining effective amounts of: a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof; a bacterial antagonist selected from the group of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) and mutants thereof; and a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal.

Yet a further embodiment of the invention is a seed assembly made by combining a plant seed with effective amounts of a spore-forming bacterial antagonist and a spore-forming fungal

antagonist. In a further embodiment, the seed is a seed of a plant selected from the group of a monocot, and a dicot. In a further embodiment, the seed is a seed of a plant selected from the group of a legume plant, and a non-legume plant. In a further embodiment, the seed is a seed of a plant selected from the group of corn, sunflower, soybean, field pea, and wheat.

5

A further embodiment of the invention is method for culturing a plant comprising: applying an antagonist disclosed herein to a seed or to the seedbed of the plant; planting the seed in the seedbed; growing the plant to yield a crop; and harvesting the crop; wherein said applying step increases the yield of the crop. In a further embodiment, the antagonist is applied to the seed or to the seedbed of a plant selected from the group of a monocot, and a dicot. In a further embodiment, the antagonist is applied to the seed or to the seedbed of a plant selected from the group of a legume plant, and a non-legume plant. In a further embodiment, the antagonist is applied to the seed or to the seedbed of a plant selected from the group of corn, sunflower, soybean, field pea, and wheat.

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Plant species that may be treated with the disclosed invention include commercial crops species, e.g., barley, oat, millet, alfalfa. The disclosed invention may also be used to treat leguminous plants (e.g., soybeans, alfalfa, and peas and non-leguminous plants (e.g., corn, wheat, and cotton). The disclosed invention may also be used to treat angiosperms and cereals.

20

Yet a further embodiment is a process comprising: making a composition by combining an essentially pure culture of *Trichoderma virens* G1-3 (ATCC 58678) with an essentially pure culture of *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) in a mixture; and

applying said composition to a seed; wherein said mixture ranges in composition from 10 to 90 percent *Trichoderma virens* G1-3 (ATCC 58678) by volume and from 90 to 10 percent *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) by volume.

5 Yet a further embodiment of the invention is a process comprising: making a composition by combining an essentially pure culture of *Trichoderma virens* G1-3 (ATCC 58678) with a plurality of essentially pure cultures of bacteria in a mixture; and applying said composition to a seed; wherein said mixture ranges in composition from 10 to 90 percent *Trichoderma virens* G1-3 (ATCC 58678) by volume.

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In one embodiment of the invention the mixture ranges in composition from 10 to 90 percent *Trichoderma virens* G1-3 (ATCC 58678) by volume and from 90 to 10 percent *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) by volume. In another embodiment of the invention, the mixture comprises about 20 percent *Trichoderma virens* G1-3 (ATCC 58678) by volume 80 percent *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) by volume. In a further embodiment of the invention, the mixture comprises about 30 percent *Trichoderma virens* G1-3 (ATCC 58678) by volume 70 percent *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) by volume. In a yet further embodiment of the invention, the mixture comprises about 40 percent *Trichoderma virens* G1-3 (ATCC 58678) by volume 60 percent *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) by volume.

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A further embodiment of the invention is an antagonist for controlling plant pathogens made by combining effective amounts of: a fungal antagonist selected from the group of a strain

of *Trichoderma virens* and mutants thereof; a bacterial antagonist selected from the group of *Bacillus subtilis* var. *amyloliquefaciens* strain TJ1000 (ATCC BAA-390) and mutants thereof; and a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal. Preferably, the strain is *Trichoderma virens* G1 21, which is presently EPA registered.

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In a further embodiment, the invention is an antagonist for controlling plant pathogens made by combining effective amounts of: a fungal antagonist selected from the group of *Trichoderma virens* G1-3 (ATCC 58678) and mutants thereof; a plurality of bacterial antagonists; and a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal. Preferably, the plurality of bacterial antagonists comprises a strain of *Erwinia carotovora* and/or a strain of *Bacillus lentimorbus*.

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In a preferred embodiment, the invention is a method comprising: combining a spore-forming fungal strain and a spore-forming bacterial strain to produce a product comprising a composition of matter disclosed herein; and applying the product to a plant or to a part of the plant; whereby application of the product produces yield enhancement in the plant.

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In another preferred embodiment, the invention is a method comprising: applying a *Trichoderma* spp. microorganism and a *Bacillus* spp. microorganism to a wettable powder to produce a combination comprising an antagonist disclosed herein; and applying the combination to a seed; whereby application of the combination produces a positive yield response in a plant growing from the seed.

In yet another preferred embodiment, the invention is a process comprising: making an agricultural inoculum disclosed herein; and applying said agricultural inoculum to a seed; wherein said agricultural inoculum ranges in composition from 1 to 99 percent *Trichoderma virens* G1-3 (ATCC 58678) by culture volume and from 99 to 1 percent *Bacillus amyloliquefaciens* TJ1000 or 1BE (ATCC BAA-390) by culture volume.

In another preferred embodiment, the invention is a composition of matter comprising: a plant seed inoculated with an agricultural inoculum disclosed herein; wherein said combination increases the yield of the plant. In another preferred embodiment, the invention is a method for increasing the yield of a plant, the method comprising: coating a seed of the plant with an effective amount of an agricultural inoculum disclosed herein; and culturing the plant.

In another preferred embodiment, the invention is a composition made by combining effective amounts of: a spore-forming fungal antagonist; and a spore-forming bacterial antagonist; wherein the spore-forming fungal antagonist does not produce a substance that substantially inhibits the growth of the spore-forming bacterial antagonist and the spore-forming bacterial antagonist does not produce a substance that substantially inhibits the growth of the spore-forming fungal antagonist; and wherein the composition is effective at increasing the yield of a plant grown from a seed to which the composition has been applied. Preferably, the composition is effective at increasing the manganese content of the plant

The compositions of the present invention can be used for controlling fungal infestations by applying an effective amount of the composition or a formulation thereof, either at one point

in time or throughout the plant/crop cycle via multiple applications. The formulation may be applied to the locus to be protected for example by spraying, atomizing, vaporizing, scattering, dusting, coating, watering, squirting, sprinkling, pouring, fumigating, and the like. The dosage of the bioagent(s) applied may be dependant upon factors such as the type of fungal pest, the carrier used, the method of application (e.g., seed, plant application or soil delivery) and climate conditions for application (e.g., indoors, arid, humid, windy, cold, hot, controlled), or the type of formulation (e.g., aerosol, liquid, or solid).

Biocontrol agents comprising the disclosed compositions may be applied in agricultural, horticultural and seedling nursery environments. This generally includes application of agents to soil, seeds, whole plants, or plant parts (including, but not limited to, roots, tubers, stems, flowers and leaves). Bio-pesticide or microbial combinations may be used alone, however, they may additionally be formulated into conventional products such as dust, granule, microgranule, pellet, wettable powder, flowable powder, emulsion, microcapsule, oil, or aerosol. To improve or stabilize the effects of the bio-pesticide, the agent may be blended with suitable adjuvants and then used as such or after dilution if necessary.

A worker skilled in the art would recognize that the bioagent(s) may be formulated for seed treatment either as a pre-treatment for storage or sowing. The seed may form part of a pelleted composition or, alternatively, may be soaked, sprayed, dusted or fumigated with the inventive compositions. Additionally, the inventive compositions may be applied to the soil or turf, a plant, crop, or a plantation. Some areas may additionally require that the invention provide for slow-release materials such that the agent is designed to have an extended release period.

In use, the invention disclosed herein may comprise the application of an aqueous or a non-aqueous spray composition to the crop. For example, the inventive composition may be applied to the soil, or to a plant part (e.g., stalk, root or leaf), or both, as an aqueous spray
5 containing spray adjuvants such as surfactants and emulsified agricultural crop oils which insure that the agent is deposited as a droplet which wets the stalk or leaf and is retained on the plant so that agent can be absorbed.

The skilled artisan would realize that the inventive compositions may be applied in
10 combination with nutrients (fertilizers) or herbicides or both, or may form part of a formulation comprising the inventive composition in combination with a fertilizer or herbicide or both. Such a formulation may be manufactured in the form of a liquid, a coating, a pellet or in any format known in the art.

15 The skilled artisan would realize that the inventive compositions may be applied to seeds as part of stratification, desiccation, hormonal treatment, or a mechanical process to encourage germination or to terminate dormancy. Treatments including the inventive agents in combination with hormones, PEG, or varying temperature, or in combination with mechanical manipulation of the seed (i.e. piercing), are contemplated.

20 Further aspects of the invention will become apparent from consideration of the drawings and the ensuing description of further embodiments of the invention. A person skilled in the art will realize that other embodiments of the invention are possible and that the details of the

invention can be modified in a number of respects, all without departing from the inventive concept. Thus, the following drawings and description are to be regarded as illustrative in nature and not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

The features of the invention will be better understood by reference to the accompanying drawings which illustrate presently further embodiments of the invention. In the drawings:

Fig. 1 is a plot that compares the incidence of stalk rot in TJ1300-treated plots versus the incidence of stalk rot in control plots.

Fig. 2 is a plot that compares final plant populations in TJ1300-treated plots versus final plant populations in control plots.

DETAILED DESCRIPTION OF THE INVENTION

A further embodiment of the invention comprises the fungus *Trichoderma virens* G1-3 (ATCC 58678). This microorganism may be obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852-1776.

A further embodiment of the invention also comprises the bacterium *Bacillus lentimorbus* TJ 1000, which is renamed herein *Bacillus amyloliquefaciens* TJ1000 or 1BE, based on a more accurate determination of the name of *Bacillus* species that occurred before this patent

application was filed. This microorganism was deposited with the ATTC on October 31, 2001, and was assigned accession number ATCC BAA-390.

A further embodiment of the invention involves combining an essentially pure culture of
5 *Trichoderma virens* G1-3 (ATCC 58678) and an essentially pure culture of *Bacillus*
amyloliquefaciens TJ1000 or 1BE (ATCC BAA-390) in a competitive culture process. The
competitive culture process involves adding the *Bacillus amyloliquefaciens* TJ1000 or 1BE
(ATCC BAA-390) to a growth medium about eight hours after the *Trichoderma virens* G1-3
(ATCC 58678) was added to the medium. The combined culture is then applied to a seed, for
10 example, a corn seed. The combination grown in a competitive culture provides protection for
seeds and plants and is especially effective in a high-stress, high-fungal pathogen environment
during the early stages of plant development.

A further embodiment of the invention involves growing an essentially pure culture of
15 *Trichoderma virens* G1-3 (ATCC 58678) and an essentially pure culture of *Bacillus*
amyloliquefaciens TJ1000 or 1BE (ATCC BAA-390) separately for five days. After the cultures
are grown separately, the compositions that contain them are combined in a 50/50 combination
by volume and then the combination is applied to a seed, for example, a corn seed. The
combined cultures are applied to a seed provides protection for seeds and plants from fungal
20 pathogens. This combination is especially effective under conditions that are less stressful to the
plant.

A further step in the process involves applying either of the above combinations to a seed involves adding an aqueous solution comprising 30 grams/liter of molasses to the solution containing the combination to produce an appropriate spore count in the resulting composition. The resulting composition is then applied to the seed as a liquid mist to achieve optimum application rates per seed using the molasses as an adhesive to adhere the spores to the seed.

In a further embodiment, the bioreactor used to culture the microorganism cultures is a New Brunswick Bioflow III bioreactor. For optimal results, the agitation setting of the bioreactor is set at about 350 rpm, the aeration setting of the bioreactor is set at about 3.0 with an aeration air pressure of about 15 pounds per square inch and the temperature setting is set at about 28 degrees Centigrade. The further growth medium for each of the individual cultures and the combined competitive culture comprises about 30 grams per liter of molasses and about 5 grams per liter of yeast extract and is referred to as a MYE medium. In A further embodiment, the medium contains about 5 milliliters of antifoam. In a further embodiment, spore production is measured by counting spores using a hemacytometer manufactured by Hausser Scientific.

A variety of seed treatments or no seed treatment may be practiced before the seed is inoculated with the disclosed inoculum. In some further embodiments, seed treatments include osmotic priming and pre-germination of the seed. Because *Trichoderma virens* G1-3 and *Bacillus amyloliquefaciens* TJ1000 or 1BE are spore formers, the disclosed inoculum does not require high moisture levels for survival and, therefore, can be applied to seed and other materials without a sticker, such as those sold under the trade names Pelgel (LipaTech), Keltrol™ (Xanthan) Cellprill or Bond.

In a further embodiment, the invention involves combining of a spore forming fungal strain and a spore forming bacterial strain to enhance ease of use and longevity of shelf life both as a stored product and when applied to a seed. In A further embodiment, the invention involves applying the disclosed *Trichoderma* microorganism and the disclosed *Bacillus* microorganism to a wettable powder, and marketing the wettable powder.

First Greenhouse Working Example

Greenhouse testing was conducted to determine the effectiveness of the disclosed biocontrol agents. Treated and untreated corn seeds were grown in soil infested with seven percent *Fusarium* infested wheat seed. In this testing, the following treatment codes were used:

CONTROL - Nothing on the seed

TJ 1000-- *Bacillus amyloliquefaciens* TJ1000 or 1BE

TJ 0300--*Trichoderma virens* G1-3

TJ 1300—50/50 combination of *Trichoderma virens* G1-3 and *Bacillus amyloliquefaciens* TJ1000 or 1BE

TJ 1310—competitive culture of *Trichoderma virens* G1-3 and *Bacillus amyloliquefaciens* TJ1000 or 1BE, resulting in a 70/30 ratio of *Trichoderma* to *Bacillus*

The results of greenhouse testing are presented in Table 0. The rating scale used was 9 = worst plant protection and 1 = best plant protection. Seed treated with biocontrol organisms grown in competitive culture showed an increase in plant protection over seed treatments with the same biological control organisms grown in non-competitive culture. The biocontrol agents were applied to the seed without a sticker.

Table 0. Greenhouse Testing Results

Treatment	Replication 1	Replication 2	Replication 3	Average
Control	9	7	6	7.3
TJ 0300	6	5	5	5.3
TJ 1000	7	6	5	6
TJ 1300	6	5	6	5.6
TJ 1310	1	3	3	2.3

Field Trials Working Example

In a subsequent experiment, field trials were conducted at seven locations throughout the U.S. Site locations included Arizona, Colorado, Kansas, Montana, North Dakota and two South Dakota locations. At each location, the trial contained a CONTROL that was treated with the industry-standard chemical treatment, MAXIM. All cultures used in the trial were grown in MYE broth for five days. *Bacillus amyloliquefaciens* TJ1000 or 1BE was cultured individually (non-competitive) and with *Trichoderma virens* G1-3 (competitive culture). *Trichoderma virens* G1-3 and *Bacillus amyloliquefaciens* TJ1000 or 1BE were also grown in non-competitive culture were also applied to the same seed to test the effectiveness of non-competitive culture versus

competitive culture. Corn seeds were treated to give a final concentration of 1,000,000,000 bacterial/fungal spores per acre. Seed treatment was done with a Gustafson benchtop seed treater, Model BLT.

5 The plot location in Kansas was severely damaged by early dry conditions and the plot was terminated prior to harvest. The Colorado location was damaged due to machine damage prior to harvest. Colorado yield data were collected but were extremely variable and were not included in the analyzed data set. The Colorado stalk rot data were included in the data set.

10 The value of the Stalk Rot variable was determined by counting ten plants in a row, determining the number of root rot/stalk rot infected plants and expressing that number as a percentage. As illustrated in Fig. 1, in six trials, the average infection rate in the control was 55.13 percent versus 38.62 percent in the entries treated with the fungal/bacterial combination, TJ1300. The data revealed an average reduction of disease incidence of 30 percent with the
15 Colorado location showing a reduction of over 60 percent.

 The value of the Final Population variable was determined by a conducting a physical count of the plants in a measured area and converting to a per acre count. As illustrated in Fig. 2, the average increase in final plant population was 3,742 plants per acre or an increase of 12.2
20 percent. This increased population was the result of controlling the disease early and having less plant death throughout the season.

Use of TJ1300 resulted in an average yield benefit of 5.35 bushels per acre. Average yield was determined from eight trials: 4 in South Dakota, 1 in North Dakota, 2 in Arizona, and 1 in Montana.

Second Greenhouse Working Example

Greenhouse Methods: All test cultures were grown in MYE (three percent Molasses, 0.5 percent Yeast Extract) broth for five days. Bacteria were grown up individually (non-competitive) and with *T. virens* G1-3 (competitive culture). *T. virens* G1-3 was also grown in a non-competitive culture for testing. *T. virens* G1-3 and test bacteria grown in non-competitive culture were also applied to the same seed to test the effectiveness of non-competitive culture versus competitive culture. Corn seeds were treated to give a final concentration of 1×10^9 bacteria/fungal spores (may also be referred to a Colony Forming Units or CFU) per acre. Seed treatment was done with a Gustafson Benchtop Seed Treater, Model BLT. Seeds were grown in soil infested with seven percent Fusarium-infested wheat seed. After four weeks, plant heights were taken as well as plant biomass. Plant heights were taken by measuring from the soil line to the tallest leaf, biomass of the plants was taken by cutting the plants at the soil line and then weighing plants on analytical scale. The treatment matrix was as follows:

Control – No pathogen added to soil.

Control – With pathogen added to soil.

TJ1000 – *Bacillus amyloliquefaciens* TJ1000 or 1BE

TJ0300 – *Trichoderma virens* G1-3

TJ2000 – *Erwinia carotovora*

TJ1300 – *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3 (non-competitive)

TJ2300 – *E. carotovora* and *T. virens* G1-3 (non-competitive)

TJ1310 – *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3 (competitive)

TJ1-2310 – *B. amyloliquefaciens* TJ1000 or 1BE , *E. carotovora* and *T. virens* G1-3

5 (competitive)

TJ2310 – *E. carotovora* and *T. virens* G1-3 (competitive)

Determination of CFU (Colony Forming Units) concentrations in competitive cultures:

Competitive cultures grown for five days. CFU counts of each organism were performed using a
 10 hemacytometer (Hausser Scientific) under light microscopy 5000x magnification. This method
 was used to determine the CFU counts in the greenhouse and field trials.

Enumeration through plate counts: Competitive cultures were grown for five days in
 submerged culture then 200 milliliters (ml) of the culture was harvested and aliquoted into four
 15 50 ml centrifuge tubes. After centrifugation at 10,000 revolutions per minute (rpm) for 10
 minutes resulting pellets were washed twice in equal volumes of D₂H₂O. Pellets were then re-
 suspended in 25 ml of saline. One ml samples were diluted 10⁻¹ to 10⁻⁸ and plated onto potato
 dextrose agar (PDA) plates. Colonies are then counted and correlated with the dilution rates to
 determine CFU per ml of culture broth.

20

Results: All of the biocontrol agents in this experiment produced significant plant
 biomass increases over the pathogen-treated control and all of the treatments were numerically
 greater than the control plants in soil that contained no pathogen. The effects of bacterial/fungal

combination TJ 1310 and the bacterial treatment TJ 1000 were significantly greater than both controls in the experiment.

Table 1. Demonstration of the Effectiveness of Biological Combinations and Individual Bacteria and Individual Fungal Treatments on Increasing the Biomass of Greenhouse-Grown Corn Seedlings in Pathogen-Treated Soil vs. the Untreated Control

Treatment	Ratio	Rank	Biomass (grams)
Control Path	0/0	10	3.62 a
Control No Path	0/0	9	7.25 ab
TJ 1300	50/50	8	8.67 b
TJ 2310	30/70	7	9.04 b
TJ 2000	100/0	6	10.73 b
TJ 1-2310	20/20/60	5	11.37 b
TJ 2300	50/50	4	11.41 b
TJ 0300	0/100	3	11.53 b
TJ 1310	30/70	2	12.24 bc
TJ 1000	100/0	1	12.89 bc
CV %			33.9
LSD (0.05)			4.55

Combinations Field Trial Working Example

Materials and Methods: A field trial was conducted using the corn variety NK 3030Bt using the following biological treatments of the seed at a rate of approximately 10^6 CFU per seed. The seed was planted at a seeding rate of 25,000 seeds per acre in 30-inch rows in a randomized, replicated block. Each entry was replicated four times. The pathogen levels were natural populations at a location near Groton, SD. The entries were as follows:

Control: Maxim Seed treatment (Maxim is a trademark of Syngenta Crop Protection)

TJ 1000 – *Bacillus amyloliquefaciens* TJ1000 or 1BE

TJ 0300 – *Trichoderma virens* G1-3

TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ 1310 – Coculture 30/70 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

5 TJ 66/300 – 50/50 combination of *Bacillus amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

10 Results: The trial produced significant yield response over the control with the entries TJ 0300, TJ 1300, and TJ 1310. The combinations TJ 1300 and TJ 1310 produced a yield response numerically greater than that of TJ 0300. The effects of bacterial/fungal combination TJ 66/300 and the bacterial treatment TJ 1000 were numerically greater than the control but not significantly greater. The results are presented in Table 2.

Conclusion: The bacterial/fungal combinations of entries TJ 1300 and TJ 1310 are the most effective biocontrol treatments in the trial for increasing the yield of corn.

15 Table 2. Effect of Biological Seed Treatment on Yield of Corn Variety N3030 Bt under Field Conditions.

Treatment	Ratio	Rank	Location	Trial	Yield
Control Maxim	0/0	6	Groton,SD	Seed Treat	164.8 a
TJ 1000	100/0	4	Groton,SD	Seed Treat	175.1 ab
TJ 0300	0/100	3	Groton,SD	Seed Treat	179.5 bc
TJ 1300	50/50	2	Groton,SD	Seed Treat	183.3 bc
TJ 1310	30/70	1	Groton,SD	Seed Treat	189.8 c
TJ 66/300	50/50	5	Groton,SD	Seed Treat	173.2 ab
CV%					13.54
LSD(0.05)					12.5

50/50 Combination Field Trial Working Example

Materials and Methods: A field trial was conducted using the corn variety NK 3030Bt using the following biological treatments of the seed at a rate of approximately 10^6 CFU per seed. The seed was planted at a seeding rate of 25,000 seeds per acre in 30-inch rows in a randomized replicated block. Each entry was replicated four times. The pathogen levels were natural populations at a location near Groton, SD. The entries were as follows:

Control: Maxim Seed treatment (Maxim is a trademark of Syngenta Crop Protection)

TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

Results: As indicated in Table 3, the trial produced a significant response in the yield of the seed treated with the biocontrol agent TJ 1300 (described above) as compared with the untreated control.

Table 3. Effect of Biological Seed Treatment on Yield of Corn Variety NK 3030Bt under Field Conditions.

Treatment	Ratio	Rep	Location	Yield
Control	0/0	1	Groton, SD	156.8
Control	0/0	2	Groton, SD	163.3
Control	0/0	3	Groton, SD	151.0
Average	0/0		Groton, SD	157.03 a
1300	50/50	1	Groton, SD	184.3
1300	50/50	2	Groton, SD	179.1
1300	50/50	3	Groton, SD	177.3
Average	50/50		Groton, SD	180.21 b
CV%				5.65
LSD (0.05%)				9.04

Application Rate Field Trial Working Example

Materials and Methods: A field trial was conducted using the corn variety NK2555 using the TJ 1300 (50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3) biological treatments of the seed at variable rates. The purpose of the trial was to identify the most effective application rate for the bacterial/fungal combination of TJ 1300. The 1x rate was approximately 1×10^6 CFU per seed. The seed was planted at a seeding rate of 25,000 seeds per acre in 30-inch rows in a randomized, replicated block. Each entry was replicated four times. The pathogen levels were natural populations at a location near Groton, SD. The entries were as follows:

Control - Maxim (Maxim is a trademark of Syngenta Crop Protection)

0.5x rate

1x rate

1.5x rate

2x rate

Results: All of the biocontrol treatments in this experiment resulted in significant yield response over the control with the 1.5x rate producing significantly better results than the 2x rate. The results of this trial, presented in Table 4, indicated that the most efficacious application rate of the biocontrol agent TJ 1300 was approximately 1.5×10^6 per seed.

Table 4. Effect of TJ1300 Biological Seed Treatment on Yield of Corn Variety N2555 at Variable Rates

Treatment	Ratio	Rank	Location	Trial	Yield
Control	0/0	5	Groton, SD	Rate	140.2 a
0.5x rate	50/50	3	Groton, SD	Rate	153.6 bc
1x rate	50/50	2	Groton, SD	Rate	156.2 bc
1.5x rate	50/50	1	Groton, SD	Rate	161.1 c
2x rate	50/50	4	Groton, SD	Rate	152.07 b
CV%					5.31
LSD (0.05%)					8.61

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Liquid Biocontrol Preparations Working Example

Materials and Methods: Field trials were conducted using the corn varieties NK 3030 and NK 3030Bt at a location in Brookings, SD and NK 3030Bt and NK2555 at a location in Groton, SD. The purpose of the trial was to compare pathogen control of liquid biocontrol preparations to a control treated with only water. The results of the trial were quantified in yield of corn in bushels per acre. The water was applied to the control at a 10 gallon per acre rate. Biocontrol treatments were prepared by adding 1×10^8 CFU per gram of a wettable powder (Mycotech, Inc.).

Two and one half grams of the wettable powder was added per one gallon of water and soil applied in the seed furrow at a rate of 10 gallons per acre. The seed was Maxim (Maxim is a trademark of Syngenta Crop Protection) treated and was planted at a seeding rate of 25,000 seeds per acre in 30-inch rows in a randomized, replicated block. Each entry was replicated four times. The pathogen levels were natural populations at each location. The entries were as follows:

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Control -- Water

TJ 1000 – *Bacillus amyloliquefaciens* TJ1000 or 1BE

TJ 0300 – *Trichoderma virens* G1-3

TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ 1310 – Coculture 30/70 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

5 TJ 66/300 – 50/50 combination of *Bacillus lentimorbus* and *T. virens* G1-3

Results: Table 5 shows a significant yield increase to the biocontrol treatments of TJ 1000, TJ1300, and TJ 66/300. All of the biocontrol treatments showed a numerical yield increase.

10 Table 6 shows a significant yield increase to the biocontrol treatments of TJ1000, TJ0300, and TJ1300. Again, all of the biocontrol treatments showed a numerical yield increase.

Table 7 shows no significance in the yield between the treatments and the control, however, the yield of TJ0300 was numerically less than the control by over 10 bushels per acre and is significantly less than the yields of the TJ1000 and TJ 1310 bacterial/fungal combination. This table demonstrates the strength of the disclosed bacterial/fungal combinations over the fungal control alone.

20 Table 8 shows the treatments of TJ 1000 and TJ 66/300 with significantly less yield than the control while the treatments of TJ0300, TJ1300, and TJ1310 having no significant difference. In this trial, it was the bacterial entry of TJ1000 alone that shows weakness in pathogen control. This table demonstrates the strength of disclosed bacterial/fungal combinations over the bacterial treatment alone.

Conclusion: The bacterial/fungal combination of entries TJ 1300 and TJ 1310 produce consistent pathogen control and/or yield response, while the bacteria entry of TJ 1000 alone and fungal entry of TJ 0300 alone produce inconsistent pathogen control and/or yield response.

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Table 5. Liquid Drench Treatment on Corn Variety NK3030 at Brookings, SD Location

Treatment	Variety	Ratio	Rank	Location	Trial	Yield
Control	NK3030	0/0	6	Brookings, SD	Liquid	162.2 a
TJ1000	NK3030	100/0	1	Brookings, SD	Liquid	179.7 b
TJ0300	NK3030	0/100	5	Brookings, SD	Liquid	170.7ab
TJ1300	NK3030	50/50	2	Brookings, SD	Liquid	177.9 b
TJ1310	NK3030	30/70	4	Brookings, SD	Liquid	172.8ab
TJ66/300	NK3030	50/50	3	Brookings, SD	Liquid	175.0 b
CV%						7.38
LSD (0..20%)						12.36

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Table 6. Liquid Drench Treatment on Corn Variety NK2555 at Groton, SD Location

Treatment	Variety	Ratio	Rank	Location	Trial	Yield
Control	NK2555	0/0	6	Groton, SD	Liquid	136.2 a
TJ1000	NK2555	100/0	1	Groton, SD	Liquid	147.7 c
TJ0300	NK2555	0/100	2	Groton, SD	Liquid	145.0bc
TJ1300	NK2555	50/50	3	Groton, SD	Liquid	142.5bc
TJ1310	NK2555	30/70	4	Groton, SD	Liquid	141.5abc
TJ66/300	NK2555	50/50	5	Groton, SD	Liquid	138.5abc
CV%						10.92
LSD (0.20%)						8.42

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Table 7. Liquid Drench Treatment on Corn Variety NK 3030Bt at Brookings, SD Location

Treatment	Variety	Ratio	Rank	Location	Trial	Yield
Control	NK3030Bt	0/0	4	Brookings, SD	Liquid	181.5 ab
TJ1000	NK3030Bt	100/0	2	Brookings, SD	Liquid	185.5 b
TJ0300	NK3030Bt	0/100	6	Brookings, SD	Liquid	171.3 a
TJ1300	NK3030Bt	50/50	5	Brookings, SD	Liquid	180.7ab
TJ1310	NK3030Bt	30/70	1	Brookings, SD	Liquid	185.8 b
TJ66/300	NK3030Bt	50/50	3	Brookings, SD	Liquid	181.6 ab
CV%						6.32
LSD (0.20%)						11.40

5 Table 8. Liquid Drench Treatment on Corn Variety 3030Bt at Groton, SD Location

Treatment	Variety	Ratio	Rank	Location	Trial	Yield
Control	NK3030Bt	0/0	2	Groton, SD	Liquid	173.9 c
TJ1000	NK3030Bt	100/0	6	Groton, SD	Liquid	164.1 a
TJ0300	NK3030Bt	0/100	4	Groton, SD	Liquid	171.3abc
TJ1300	NK3030Bt	50/50	3	Groton, SD	Liquid	171.5abc
TJ1310	NK3030Bt	30/70	1	Groton, SD	Liquid	176.3 c
TJ66/300	NK3030Bt	50/50	5	Groton, SD	Liquid	164.4 ab
CV%						10.92
LSD (0.20%)						8.42

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Compatibility with Dry Granule Micro-Nutrient Fertilizer Working Example

15 Materials and Methods: A field trial was conducted using the corn variety NK 3030Bt at a location in Groton, SD. The purpose of the trial was to compare the compatibility and yield benefit of the biocontrol preparation TJ1300 in combination with a dry granule micro-nutrient fertilizer vs. the micro-nutrient fertilizer alone vs. a control with no micro-nutrient fertilizer. The micro-nutrient fertilizer is sold commercially by the applicant under the trademark TJ

20 Micromix[™]. Biocontrol treatments were prepared by adding 1×10^6 CFU per seed. The control

seed was Maxim (Maxim is a trademark of Syngenta Crop Protection) treated with the biocontrol treatments applied in addition to the Maxim. The seed was planted at a seeding rate of 25,000 seeds per acre in 30-inch rows in a randomized, replicated block. TJ Micromix[™] was applied at a rate of 20 pounds per acre. Each entry was replicated four times. The pathogen levels were natural populations at each location. The entries were as follows:

Control: Maxim

TJ Micromix

TJ Micromix + TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

Results: In this trial, as shown in Table 9, the Granular TJ Micromix produced a non-significant yield increase compared to the control. When the seed-applied biocontrol treatment TJ1300 was applied in combination with the TJ Micromix, the treatment resulted in a significant increase in yield.

Conclusion: The trial shows that TJ 1300 is compatible with micro-nutrient applications and the combination produces a significant yield response.

Table 9. Effect of TJ Micromix and TJ Micromix + TJ 1300 on Corn Variety NK 3030Bt

Treatment	Variety	Rank	Location	Trial	Yield
Control	NK3030Bt	3	Groton, SD	Fertilizer	157.0 a
TJ Micromix	NK3030Bt	2	Groton, SD	Fertilizer	163.3 ab
TJ Micromix + TJ 1300	NK3030Bt	1	Groton, SD	Fertilizer	175.5 b
CV%					9.04
LSD (0.05%)					5.64

Compatibility with Liquid Chelate Micro-Nutrient Fertilizer Working Example

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Materials and Methods: A field trial was conducted using the corn variety NK 3030Bt at a location in Groton, SD. The purpose of the trial was to compare the compatibility and yield benefit of the biocontrol preparation TJ1300 in combination with a liquid chelate micro-nutrient fertilizer vs. the liquid chelate micro-nutrient fertilizer alone. The liquid chelate micro-nutrient fertilizer is sold commercially under the Trademark TJ Micromixtm - Cornmix. Biocontrol treatments were prepared by adding 1×10^6 CFU per seed. The control seed was Maxim (Maxim is a trademark of Syngenta Crop Protection) treated with the biocontrol treatments applied in addition to the Maxim. The seed was planted at a seeding rate of 25,000 seeds per acre in 30-inch rows in a randomized, replicated block. TJ Micromixtm - Cornmix was applied at a rate of 1.5 quarts per acre. Each entry was replicated four times. The pathogen levels were natural populations at the location. The entries were as follows:

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Control: Maxim + Liquid Chelate TJ Micromix

TJ Micro + TJ1000: Liquid Chelate TJ Micromix plus TJ 1000 - *B. amyloliquefaciens* TJ1000 or 1BE

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TJ Micro + TJ0300: Liquid Chelate TJ Micromix plus TJ 0300 - *T. virens* G1-3

TJ Micro + TJ1300: Liquid Chelate TJ Micromix + TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ Micro + TJ1310: Liquid Chelate TJ Micromix + TJ 1310 – Coculture 30/70 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ Micro + TJ66/300: Liquid Chelate TJ Micromix + TJ 66/300 – 50/50 combination of *Bacillus lentimorbus* and *T. virens* G1-3

Results: As shown in Table 10, the biocontrol treatments TJ1000, 66/300, and 1300 combined with the liquid chelate TJ Micromix resulted in a significant increase in yield over the control of TJ Micromix alone. The other biocontrol entries showed numerical but non-significant increases in yield. The conclusion was that the biocontrol agents used in this study are compatible with liquid chelate micro-nutrient applications. This biocontrol/liquid chelate micro-nutrient fertilizer combination is a viable means to significantly increase the yield of corn.

Table 10. Effect of TJ Micromix Liquid Chelate and TJ Micromix Liquid Chelate + TJ 1300 on Yield of Corn Variety NK3030Bt

Treatment	Variety	Ratio	Rank	Location	Trial	Yield
Control	NK3030Bt	0/0	6	Groton, SD	Liquid TJ Micromix	161.0 a
TJ Micro + TJ 1000	NK3030Bt	100/0	3	Groton, SD	Liquid TJ Micromix	173.0 bc
TJ Micro + TJ 0300	NK3030Bt	0/100	5	Groton, SD	Liquid TJ Micromix	163.0 ab
TJ Micro + TJ1300	NK3030Bt	50/50	1	Groton, SD	Liquid TJ Micromix	183.7 c
TJ Micro + TJ 1310	NK3030Bt	30/70	4	Groton, SD	Liquid TJ Micromix	172.0 ab
TJ Micro + TJ 66/300	NK3030Bt	50/50	2	Groton, SD	Liquid TJ Micromix	173.2 bc
CV%						11.2
LSD (0.05%)						12.36

Sunflower Dry Granule Micro-Nutrient Fertilizer Working Example

Materials and Methods: A field trial was conducted using the sunflower variety Pioneer 63M80 NuSun at a location in Hazelton, ND. The purpose of the trial was to compare the compatibility and yield benefit of the biocontrol preparation TJ1300 in combination with a dry granule micro-nutrient fertilizer vs. the micro-nutrient fertilizer alone vs. a control with no micro-nutrient fertilizer. Analyzing yield of sunflower is a function of seed yield in pounds per acre and the amount of oil in the seed which is expressed as a percentage. The micro-nutrient fertilizer is sold commercially under the Trademark TJ Micromix™. Biocontrol treatments were prepared by adding 1×10^6 CFU per seed. The control seed was Maxim (Maxim is a trademark of Syngenta Crop Protection) treated with the biocontrol treatments applied in addition to the Maxim. The seed was planted at a seeding rate of 22,000 seeds per acre in 30-inch rows in a randomized, replicated block. TJ Micromix™ was applied at a rate of 20 pounds per acre. Each entry was replicated four times. The pathogen levels were natural populations at the location. The entries were as follows:

Control: Maxim

TJ Micromix

TJ 1300 - 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ Micromix + TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

Results: As shown in Table 11, in this trial, the Granular TJ Micromix produced a significant yield increase and a significant oil percentage increase compared to the control. When the seed-applied biocontrol treatment TJ1300 was applied in combination with the TJ Micromix, the treatment resulted in a significant increase in yield as compared to the control but not significantly different from the TJ Micromix application alone. The yield of the TJ 1300 +

TJ Micromix was numerically higher in yield. The conclusion was that TJ 1300 is compatible with micro-nutrient applications and may be a viable tool to increase the yield of sunflower.

5 Table 11. Effect of TJ1300 Liquid Biological Treatment Plus Dry Granular TJ Micromix on Yield of Nu-sun Sunflower Variety 63M80

Treatment	Rank	Location	Trial	Yield	Oil
Control		Hazelon, ND	TJ Micro	1709.7 a	44.8 a
TJ Micromix		Hazelon, ND	TJ Micro	1857.3 bc	47.2 b
TJ 1300		Hazelon, ND	TJ Micro	1734.7ab	45.5 a
TJ 1300 + TJ Micromix		Hazelon, ND	MM	1864.7 bc	44.9 a
CV%				7.48	4.67
LSD (0.20)				132.8	1.5

Sunflower Liquid Chelate Micro-Nutrient Working Example

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Materials and Methods: Field trial was conducted using the sunflower variety Pioneer 63M80 NuSun at 3 locations: Hazelton, ND; Kensal, ND; and Selby, SD. The purpose of each trial was to compare the compatibility and yield benefit of the biocontrol preparation TJ1300 in combination with a liquid chelate micro-nutrient fertilizer vs. an untreated control. Analyzing yield of sunflower is a function of seed yield in pounds per acre and the amount of oil in the seed which is expressed as a percentage. The liquid chelate micro-nutrient fertilizer is sold commercially under the Trademark TJ Micromix[™]. Biocontrol treatments were prepared by adding 1×10^8 CFU per gram to a wettable powder (Mycotech, Inc). 25 grams of the wettable powder was then added to 1.5 quarts of liquid chelate TJ Micromix and the combination applied

in the seed furrow at a rate of 1.5 quarts per acre. The control seed was Maxim (Maxim is a trademark of Syngenta Crop Protection) treated with the biocontrol treatments applied in addition to the Maxim. The seed was planted at a seeding rate of 22,000 seeds per acre in 30- inch rows in a randomized, replicated block. Each entry was replicated four times. The pathogen levels were natural populations at each location. The entries were as follows:

Control - no treatment

TJ 1300 - 50/50 combination of *B. amyloliquefaciens* GL-3 and *T. virens* G1-3

TJ1300 + TJ Micromix - Liquid chelate TJ Micromix + 50/50 combination of *B. amyloliquefaciens* and *T. virens*

Result: As shown in Table 12, TJ Micromix liquid and the combination of TJ Micromix plus TJ 1300 both gave sunflower a significant increase in yield. TJ 1300 + TJ Micromix produced an additional numerical increase in yield over the TJ Micromix alone.

Conclusion: TJ 1300 + TJ Micromix is a viable means of biocontrol delivery on sunflower and is a viable means of increasing the seed yield of sunflower.

Table 12. Effect of TJ1300 Biological Liquid Plus Liquid TJ Micromix Fertilizer on Yield of Nu-sun Sunflower Variety 63M80

Treatment	Ratio	Location	Trial	Yield	Oil
Control	0/0	Hazelton, ND	Liquid TJ Micro	1709.7	44.8
TJ 1300	50/50	Hazelton, ND	Liquid TJ Micro	1765.0	45.5
TJ1300+TJ Micromix	50/50	Hazelton, ND	Liquid TJ Micro	1992.3	45.9
Control	0/0	Kensal, ND	Liquid TJ Micro	2000.3	N/a

TJ1300	50/50	Kensal, ND	Liquid TJ Micro	2159.0	N/a
TJ1300+TJ Micromix	50/50	Kensal, ND	Liquid TJ Micro	2329.0	N/a
Control	0/0	Selby, SD	Liquid TJ Micro	2225.0	43.2
TJ 1300	50/50	Selby, SD	Liquid TJ Micro	2324.0	44
TJ1300 + TJ Micromix	50/50	Selby, SD	Liquid TJ Micro	2228.5	44
Control Average				1978.3 a	44 a
TJ 1300				2082.8 b	44.75 a
TJ 1300 + TJ Micromix				2173.3 b	45.5 a
CV%				10.58	4.67
LSD (0.05)				104.1	NS

Soybean Liquid Chelate Micro-Nutrient Fertilizer Working Example

5 **Materials and Methods:** A field trial was conducted using the soybean variety Pioneer 91B52 a location near Groton, SD. The purpose of the trial was to compare the compatibility and yield benefit of the biocontrol preparation TJ1300 in combination with a liquid chelate micro-nutrient fertilizer vs. the liquid chelate alone vs. an untreated control. Yield in bushels per acre was used as the measure of the treatment response. The liquid chelate micro-nutrient fertilizer is sold commercially under the Trademark TJ Micromix[™]. Biocontrol treatments were prepared by adding 1×10^8 CFU per gram to a wettable powder (Mycotech, Inc). Twenty-five grams of the wettable powder was then added to 10 gallons of water and applied in the seed furrow at a rate of 10 gallons per acre to establish treatment TJ1300. Twenty-five grams of the wettable powder was added to 1.5 quarts of liquid chelate TJ Micromix and the combination added to water to form a 10 gallon solution and applied in the seed furrow at a rate of 10 gallons per acre. The

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seed was planted at a seeding rate of 175,000 seeds per acre in 30-inch rows in a randomized, replicated block. Each entry was replicated four times. The pathogen levels were natural populations at the location. The entries were as follows:

Control - no treatment

5 TJ 1300 - 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ1300 + TJ Micromix - Liquid chelate TJ Micromix + 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

10 Result: As shown in Table 13, TJ Micromix liquid and the combination of TJ Micromix plus TJ 1300 both gave soybean a significant increase in yield. TJ 1300 + TJ Micromix produced an additional numerical but non significant increase in yield over the TJ Micromix alone.

Conclusion: TJ 1300 + TJ Micromix is a viable means of biocontrol deliver on soybean and is a viable means of increasing the yield of soybean.

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Table 13. Effect of TJ1300 Liquid Biological Treatment Plus Liquid TJ Micromix Fertilizer on Yield of Soybean Variety 91B52

Treatment	Ratio	Location	Trial	Yield
Control	0/0	Groton, SD	Liquid TJ Micromix	54.2 a
TJ 1300	50/50	Groton, SD	Liquid TJ Micromix	60.8 b
TJ1300 + TJ Micromix	50/50	Groton, SD	Liquid TJ Micromix	61.8 b
CV%				8.92
LSD (0.05)				4.19

Soybean Dry Granule Micro-Nutrient Working Example

Materials and Methods: A field trial was conducted using the soybean variety Pioneer 91B52 at a location near Groton, SD. The purpose of the trial was to compare the compatibility and yield benefit of the biocontrol preparation TJ1300 in combination with a dry granule micro-nutrient fertilizer vs. the micro-nutrient fertilizer alone vs. a control with no micro-nutrient fertilizer. Soybean yield in bushels per acre was used to measure the treatment response. The micro-nutrient fertilizer is sold commercially under the Trademark TJ Micromix™. Biocontrol treatments were prepared by adding 1×10^5 CFU per seed. The seed was planted at a seeding rate of 175,000 seeds per acre in 30-inch rows in a randomized, replicated block. TJ Micromix™ was applied at a rate of 20 pounds per acre. Each entry was replicated four times. The pathogen levels were natural populations at each location. The entries were as follows:

Control: Maxim

TJ Micromix

TJ 1300 - 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

TJ Micromix + TJ 1300 – 50/50 combination of *B. amyloliquefaciens* TJ1000 or 1BE and *T. virens* G1-3

Results: As shown in Table 14, in this trial, the Granular TJ Micromix produced a significant yield increase compared to the control. When the seed-applied biocontrol treatment TJ1300 was applied in combination with the TJ Micromix, the treatment resulted in a significant

increase in yield as compared to the control but not significantly different from the TJ Micromix application alone. The yield of the TJ 1300 + TJ Micromix was numerically higher.

Conclusion: TJ 1300 is compatible with micro-nutrient applications and is a viable tool to increase the yield of soybean.

5 **Table 14.** **Effect of TJ1300 Biological Seed Treatment Plus Dry Granule TJ Micromix Fertilizer on Yield of Soybean Variety 91B52**

Treatment	Ratio	Location	Trial	Yield
Control	0/0	Groton, SD	TJ Micro	54.2 a
TJ Micromix Granule	0/0	Groton, SD	TJ Micro	61.6 b
TJ 1300	50/50	Groton, SD	TJ Micro	62.5 b
TJ 1300 + TJ Micromix	50/50	Groton, SD	TJ Micro	63.3 b
CV%				8.92
LSD (0.05)				4.19

Spring Wheat Working Example

10 **Materials and Methods:** A field trial was conducted using Russ Spring wheat at a location near Kensal, ND. The purpose of the trial was to test biocontrol TJ 1300 on spring wheat against an untreated control. The biocontrol TJ 1300 was applied to the seed so as to achieve an application rate of 2.5×10^9 CFU per acre. The plot was planted in a randomized, replicated block design with each entry replicated three times.

15 **Result:** As shown in Table 15, the entry TJ 1300 produced a non-significant yield increase. The conclusion was that TJ 1300 may be of value as a seed treatment on wheat.

Table 15. **Effect of TJ1300 Biological Seed Treatment Plus Fertilizer on Russ Spring Wheat**

Treatment	Ratio	Location	Trial	Yield
Control	0/0	Kensal, ND	MM	43.8
1300	50/50	Kensal, ND	MM	44.0
CV%				7.52
LSD (0.05)				NS

Field Peas Working Example

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Materials and Methods: A field trial was conducted to compare the biocontrol treatment TJ 1300 to a non-treated control on field peas. The seed was treated with the biocontrol agent to achieve an application of 2.5×10^9 CFU per acre. Yield response was measured as pounds per acre.

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Results: As shown in Table 16, the entry TJ 1300 produced a non-significant yield increase in field peas. The conclusion was that TJ 1300 may be an effective tool to increase the yield of field peas.

15 Table 16. Effect of TJ1300 Biological Seed Treatment on Yield of Integra Field Pea

Treatment	Ratio	Rep	Location	Trial	Yield	Test weight
Control	0/0	Ave of 3	Carrington, ND	Pea	3590.0	62.9
1300	50/50	Ave of 3	Carrington, ND	Pea	3613.0	63.5
CV %					7	0.5
LSD (0.05)					ns	Ns

5 Increased Manganese Uptake Working Example

A surprising aspect of the subject invention is that plants that grow from seeds treated with the disclosed combination experience increased uptake of manganese. The protective nature of increased manganese uptake is documented in Project S-269: Biological Control and
10 Management of Soilborne Plant Pathogens for Sustainable Crop Production, 5th International Conference on the Biogeochemistry of Trace Elements. July 11-15 1999. Vienna, Austria, p. 1086-1087. Dr. Don Huber of Purdue University has documented the connection between an imbalance in the ratio of nitrogen to manganese and the incidence of stalk rot in corn. (Huber D. 2000. "Hidden Hunger" threatens many crops. Purdue News.

15 The disclosed combination of *Trichoderma virens* and *Bacillus amyloliquefaciens* for the purpose of plant pathogen control and increased plant yield thus has unexpected characteristics. The first is the fact that the combination produces an increase in yield, not just plant protection
20 from the pathogen. Plant tissue analysis from test plots presented in Tables 17 and 18 below show an unexpected trend toward higher nutrient intake of a nutrient, manganese.

The treatments that produced the surprising results shown in Table 17 are defined as follows:

bs-unt-bt = Brookings, SD location – no treatment on the seed – Bt variety of corn

bs-max-bt = Brookings, SD location –chemical fungicide Maxim on the seed –Bt variety of corn

5 bs-1000-bt = Brookings, SD location –*Bacillus amyloliquefaciens* TJ 1000 on the seed– Bt variety

bs-0300-bt=Brookings, SD location-*Trichoderma virens* G1-3 on the seed – Bt variety of corn

bs-1300-bt = Brookings, SD location- *B. amyloliquefaciens* TJ 1000 and *T. virens* G1-3 (1 to 1 ratio) on the seed – Bt variety of corn (one of the claimed combinations)

10 bs-1310-bt = Brookings, SD location – *B. amyloliquefaciens* TJ 1000 and *T. virens* G1-3 (7 to 3 ratio) on the seed – Bt variety of corn

bs-66/300-bt = Brookings, SD location – *B. lentimorbus* and *T. virens* G1-3 (1 to 1 ratio) on the seed – Bt variety of corn

15 The term “Bt” is defined as: A corn hybrid that has been genetically modified by the insertion of a gene from the bacteria *Bacillus thuringiensis*. The inserted gene produces a protein that will kill European corn bore that feed on the plant tissue.

Table 17. Effects of Treatments on Plant Mineral Content on Bt Variety of Corn at Brookings SD Location

Treatment	Concentration											
	N	P	K	Mg	Ca	S	Na	Fe	Mn	B	Cu	Zn
bs-unt-bt	3.43	0.39	1.65	0.66	1.11	0.29	0.003	110	105	17	18	32

bs-max-bt	3.42	0.43	2.10	0.56	0.91	0.27	0.005	117	91	14	18	29
bs-1000-bt	3.44	0.40	2.10	0.52	0.86	0.24	0.004	96	91	12	13	25
bs-300-bt	3.38	0.41	2.02	0.58	1.00	0.27	0.004	97	98	12	14	25
bs-1300-bt	3.36	0.43	1.89	0.66	1.11	0.27	0.004	118	134	13	16	28
bs-1310-bt	3.45	0.41	1.69	0.59	1.02	0.25	0.004	182	106	16	15	27
bs-66/300-bt	3.30	0.42	2.19	0.58	1.04	0.27	0.004	112	107	16	15	29

The treatments that produced the surprising results in Table 18 are defined as follows:

bl-unt-non = Brookings location – no treatment on the seed – non Bt variety of corn (non Bt can also be described as: non genetically modified)

5 bl-max-non = Brookings location – chemical fungicide Maxim on the seed – non Bt variety of corn

bl-1000-non = Brookings location – *Bacillus amyloliquefaciens* TJ 1000 on the seed – non Bt variety of corn

bl-300-non = Brookings location – *Trichoderma virens* G1-3 on the seed – non Bt variety of corn

10 bl-1300-non = Brookings location – *B. amyloliquefaciens* TJ 1000 and *T. virens* G1-3 on the seed (1 to 1 ratio) – non Bt variety of corn (one of the claimed combinations)

bl-1310-non = Brookings location – *B. amyloliquefaciens* TJ 1000 and *T. virens* G1-3 on the seed (7 to 3 ratio) – non Bt variety of corn

15 bl-66/300-non = Brookings location – *B. lentimorbus* and *T. virens* G1-3 on the seed (1 to 1 ratio) – non Bt variety of corn

Table 18. Effects of Treatments on Plant Mineral Content on Non Bt Variety of Corn at Brookings SD Location

Treatment	Concentration											
	N	P	K	Mg	Ca	S	Na	Fe	Mn	B	Cu	Zn
bl-unt-non	3.33	0.39	1.93	0.55	0.85	0.21	0.005	76	103	12	13	24
bl-max-non	3.28	0.48	2.39	0.62	0.92	0.24	0.007	101	116	12	15	28
bl-1000-non	3.14	0.51	2.39	0.64	0.95	0.25	0.008	103	115	12	15	26
bl-300-non	3.19	0.48	2.21	0.65	0.93	0.24	0.009	95	99	15	15	24
bl-1300-non	3.38	0.48	2.43	0.60	0.96	0.25	0.006	111	137	13	15	26
bl-1310-non	3.21	0.46	2.18	0.68	1.03	0.26	0.007	108	117	18	16	25
bl-66/300-non	3.23	0.43	1.96	0.61	0.86	0.23	0.009	93	95	11	13	25

5 Manganese is known in the art as a disease prevention micronutrient. However, if manganese is added to fertilizer and applied to corn, the expected result is a decrease in yield. The significance of the subject invention is that it increases the manganese content of the corn plant while increasing yield. Furthermore, the increase in the manganese content in the plant does not occur with either organism alone or when the *Trichoderma virens* is combined with a

10 different organism (e.g., treatment 66/300) or the formulation of the mixture is altered (e.g., treatment 1310). This increase in manganese content of the plant tissue is documented in tables 1 and 2 above on Bt (genetically modified) corn and conventional (non-genetically modified) corn. Tissue analysis of the corn in the charts above was done after the silking and pollination of the corn, documenting that this increase in manganese continues into the late stages of growth.

Late season intake is significant because the lack of manganese in the plant is implicated in mid to late season stalk rot.

Data from disclosed combinations of the Trichoderma with other bacteria strains show that other combinations tested did not increase the manganese levels to the level of the present invention. It is surprising that neither organism alone increased the manganese level in the tissue of the corn. Only seed treatment with the claimed combination of the *T. virens* G1-3 fungus and the *B. amyloliquefaciens* bacterium increase the manganese level in the tissue of both the Bt and non-Bt corn.

Consistency of Increased Yield Working Example

Another surprising aspect of the subject invention is unexpected consistency of increased yield: (1) consistency compared to either organism alone, in that our field trial results show the claimed combination to be significantly higher in yield over the control in both individual locations and multiple location and either organism alone did not produce a significant yield response over the control; (2) consistency across geography, in that the field trial results show the combination to be effective in a number of geographies from North Dakota to Arizona; and (3) consistency of higher yield in a more than one crop, in that the field data collected on corn, soybeans, sunflowers and wheat show significant increased in yield with the claimed combination. Field trial results are presented in the above working examples. The results of those field trials produced a surprisingly consistent yield response, and consistency is what is commercially important.

The disclosed combination of microorganisms gives more consistent yield response than either microorganism alone. The claimed combination produces a consistent increase in yield over a range of conditions while alone the microorganisms do not. The data in the patent application show this, but the data presented in Table 19 below that was produced at the experiment station in Carrington, ND show this effect.

Table 19. Consistency of Yield Response

	2000	2001	2002	3 YR
Control	96.9	146	87.7	110.2
Bacillus	93.3	150	94.9	112.7
T. virens	94.7	162	88.5	115.1
QuickRoot	105.6	156	90.4	117.3
1310	89.5	151	88.5	109.6

In Table 19, the treatments are defined as follows:

Control = chemical fungicide Maxim

Bacillus = *B. amyloliquefaciens* alone

T. virens = *T. virens* G1-3 alone

Quick Root = QuickRoots™ is the product name of the claimed combination of *T. virens* G1-3 and *B. amyloliquefaciens*

1310 = *T. virens* G1-3 and *B. amyloliquefaciens* at a 7:3 ratio.

The column headings in Table 19 denote the year of the trial with “3YR” indicating the average treatment response for the combined three years. Note that in 2000, seed treatment with the individual organisms alone (the individual components of the claimed combination) produced yields that were less than control. In 2001, seed treatment with individual organisms both
5 produced yields that were greater than the control as did the claimed combination. In 2002, seed treatment with the individual organisms produced yields that were greater than the control and again the claimed combination increased yield as well.

The North Dakota data presented in Table 19 document consistency in two of ways.
10 First, in reviewing year 2000 data, neither the Bacillus bacteria (1000) seed treatment nor the Trichoderma fungi (G1-3) seed treatment by themselves produced a positive yield response; but the claimed combination did produce a positive response. Two negative responses added together do not produce a positive. Synergism is what creates positive response from two negatives. In years 2001 and 2002, the performance of treatments with the bacteria and the fungi
15 traded places as the top seat while the performance of the claimed combination performed between treatments with the individual components. Overall, the consistent performance of the claimed combination gave the largest yield advantage because of consistency of response. These data are from the same location; only weather changed from season to season. The Bacillus alone seed treatment did not perform well at all in the average and the Trichoderma alone seed
20 treatment only averaged well because it had one great performance out of three.

Presented in Table 20 is a compilation of data from three years of field trials, 63 entries, at 12 locations. The test plots were located at North Dakota State University, University of

Arizona, and Colorado State University. This compilation clearly shows the 50/50 combination of *B. amyloliquefaciens* + *T. virens* (one of the claimed combinations) produces a significantly higher yield than the control and than either organism alone. It should be noted that while the individual components show a numerical increase in yield, it is a non-significant increase at a 0.05 rejection level while the claimed combination is significant at a 0.05 rejection level.

Table 20. QuickRoots™ Effect on Corn Yield in Replicated Field Trials.

3 Year Average Evaluating QuickRoots™/Maxim vs. Maxim

Treatment	Moisture	Yield	Pricing	Advantage
Control	17.5	154.77	\$300.25	
<i>B. amyloliquefaciens</i> alone	17.5	158.7	\$307.88	\$7.62
<i>T. virens</i> alone	17.4	158.81	\$308.57	\$8.31
<i>B. amyloliquefaciens</i> + <i>T. virens</i> combined 50/50	17.5	161.62	\$313.54	\$13.29
Mean	17.5	158.88	\$307.56	
CV (%)	23.3	21.7		
LSD (0.05)	.19(NS)	5.05		

Many variations of the invention will occur to those skilled in the art. Some variations include non-competitive culturing of the biocontrol organisms. Other variations call for

competitive culturing. All such variations are intended to be within the scope and spirit of the invention.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An agricultural inoculum suitable for inoculating plant seeds, said inoculum comprising:
Trichoderma virens isolate G1-3 (ATCC 58678);
Bacillus amyloliquefaciens strain TJ1000 (ATCC BAA-390); and
a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal.
2. A method of protecting a plant from disease caused by a plant pathogenic fungus comprising:
inoculating seeds from said plant with an effective amount of;
Trichoderma virens G1-3 (ATCC 58678) and
Bacillus amyloliquefaciens TJ1000 (ATCC BAA-390),
wherein said combination suppresses growth of plant pathogenic fungi.
3. A method of protecting a seed or a plant from disease caused by a plant pathogenic fungus comprising:
inoculating seeds from said plant with a composition comprising a fungal antagonist and a bacterial antagonist, wherein said fungal antagonist is *Trichoderma virens*; and wherein said bacterial antagonist is *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390).
4. A method of protecting a seed or a plant from disease caused by a plant pathogenic fungus comprising:

inoculating seeds from said plant with a composition comprising the agricultural inoculum of claim 1,

wherein said inoculum suppresses growth of plant pathogenic fungi.

5. The method of claim 4 wherein the inoculum suppresses growth of the plant pathogen fungi *Fusarium*, *Phythium*, *Phytophthora* and *Penicillium*.

6. A method of protecting a plant from disease caused by a plant pathogenic fungus comprising:

inoculating said plant with a composition comprising *Trichoderma virens* isolate G1-3 (ATCC 58678) and *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390),

wherein said combination suppresses growth of plant pathogenic fungi.

7. A method for biologically controlling or inhibiting stalk rot or root rot comprising:

coating seeds with an effective amount of a composition comprising *Trichoderma virens* G1-3 (ATCC 58678) and *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390).

8. A process for making a composition comprising:

introducing a culture of *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390) to a growth medium about eight hours after a culture of *Trichoderma virens* G1-3 (ATCC 58678) is introduced to the growth medium; and

growing the culture as a competitive culture.

9. A process comprising making a composition comprising:
combining a culture of *Trichoderma virens* G1-3 (ATCC 58678) with a culture of *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390) in a 50:50 mixture; and
applying said composition to a seed at a rate of at least 100,000 spores per seed.
10. A method for protecting plants in a growing medium from damping off and root rot fungal plant disease comprising:
placing in the growing medium in the immediate vicinity of the plant to be protected or in contact with the plant to be protected an effective quantity of the agricultural inoculum of claim 1.
11. A method for protecting plants from fungal plant disease comprising:
adding the agricultural inoculum of claim 1 in an effective quantity to a substrate selected from the group of pelletized calcium sulfate and pelletized lime; and
placing the pellet in the immediate vicinity of the plant to be protected or in contact with the plant to be protected.
12. The method of claim 11 further comprising:
adding another plant growth nutrient to the pellet.
13. A method for protecting a plant from fungal plant disease comprising:
adding the agricultural inoculum of claim 1 in an effective quantity to a liquid solution;
and

applying the liquid solution in the immediate vicinity of the plant to be protected or in contact with the plant to be protected.

14. The method of claim 13 further comprising:

adding an additive to the liquid, said additive being at least one substance selected from the group of

a plant nutrient,

a plant micro-nutrient, and

a chemical fungicide.

15. A method for biologically controlling a plant disease caused by a plant-colonizing fungus, the method comprising:

inoculation of a seed of the plant with an effective amount of a microbial inoculant comprising a combination having all of the identifying characteristics of the agricultural inoculum of claim 1, said inoculation resulting in the control of said plant disease.

16. The method of claim 15, wherein said inoculation results in the control of more than one plant disease.

17. A composition comprising:

Trichoderma virens isolate G1-3 (ATCC 58678); and

Bacillus amyloliquefaciens strain TJ1000 (ATCC BAA-390).

18. An antagonist for controlling plant pathogens comprising:

Trichoderma virens isolate G1-3 (ATCC 58678);

Bacillus amyloliquefaciens strain TJ1000 (ATCC BAA-390); and

a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal.

19. A method for culturing a plant comprising:

applying the antagonist of claim 18 to a seed or to the seedbed of the plant;

planting the seed in the seedbed;

growing the plant to yield a crop; and

harvesting the crop,

wherein said applying step increases the yield of the crop.

20. The method of claim 19 wherein the antagonist is applied to the seed or to the seedbed of a plant selected from the group of

a monocot, and

a dicot.

21. The method of claim 19 wherein the antagonist is applied to the seed or to the seedbed of a plant selected from the group of

a legume plant, and

a non-legume plant.

22. The method of claim 19 wherein the antagonist is applied to the seed or to the seedbed of a plant selected from the group of

corn,

sunflower,

soybean,

field pea, and

wheat.

23. A process comprising:

making a composition by combining a culture of *Trichoderma virens* G1-3 (ATCC 58678) with a culture of *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390) in a mixture; and

applying said composition to a seed, wherein said mixture ranges in composition from 10 to 90 percent *Trichoderma virens* G1-3 (ATCC 58678) by culture volume and from 90 to 10 percent *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390) by culture volume.

24. An antagonist for controlling plant pathogens made by combining effective amounts of:

a strain of *Trichoderma virens* and

Bacillus amyloliquefaciens strain TJ1000 (ATCC BAA-390).

25. The antagonist of claim 24, further comprising a suitable carrier that is non-phytotoxic, non-bacteriostatic, and non-bactericidal.

26. The antagonist of claim 24 or 25, wherein the strain is *Trichoderma virens* G1-21.

27. A method comprising:

combining a spore-forming fungal strain and a spore-forming bacterial strain to produce a product comprising the composition of claim 17; and

applying the product to a plant or to a part of the plant,

whereby application of the product produces yield enhancement in the plant.

28. A method comprising:

applying *Trichoderma virens* isolate G1-3 (ATCC 58678) and *Bacillus amyloliquefaciens* strain TJ1000 (ATCC BAA-390) to a wettable powder to produce a combination comprising the antagonist of claim 18; and

applying the combination to a seed,

whereby application of the combination produces a positive yield response in a plant growing from the seed.

29. A process comprising:

making the agricultural inoculum of claim 1; and

applying said agricultural inoculum to a seed,

wherein said agricultural inoculum ranges in composition from 1 to 99 percent *Trichoderma virens* G1-3 (ATCC 58678) by culture volume and from 99 to 1 percent *Bacillus amyloliquefaciens* TJ1000 (ATCC BAA-390) by culture volume.

30. A method for increasing the yield of a plant, the method comprising:
coating a seed of the plant with an effective amount of the agricultural inoculum of claim 1; and
culturing the plant.
31. A composition made by combining effective amounts of:
Trichoderma virens isolate G1-3 (ATCC 58678); and
Bacillus amyloliquefaciens TJ1000 (ATCC BAA-390),
wherein the composition is effective at increasing the yield of a plant grown from a seed to which the composition has been applied.
32. The composition of claim 31 wherein the composition is effective at increasing the manganese content of the plant.

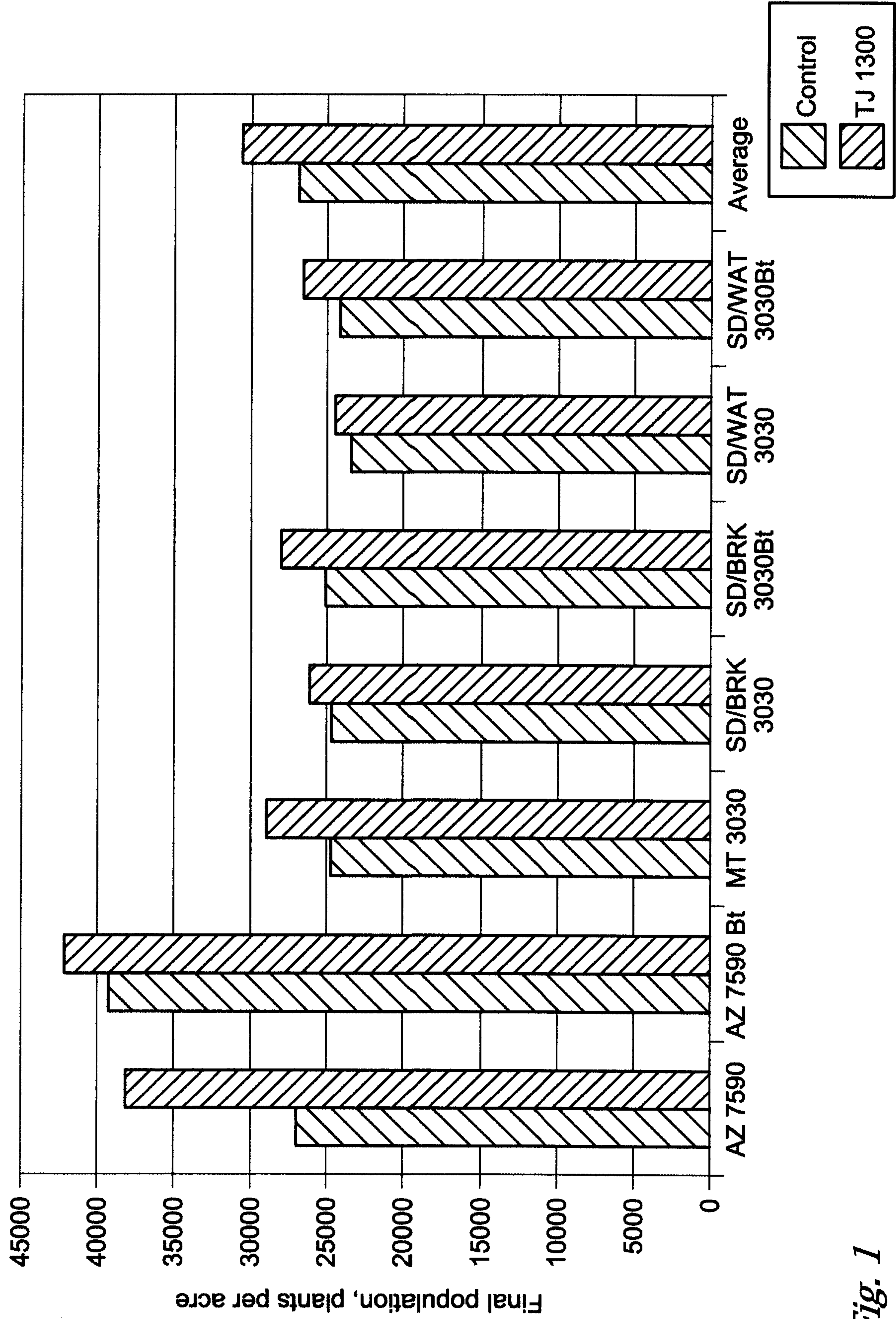


Fig. 1

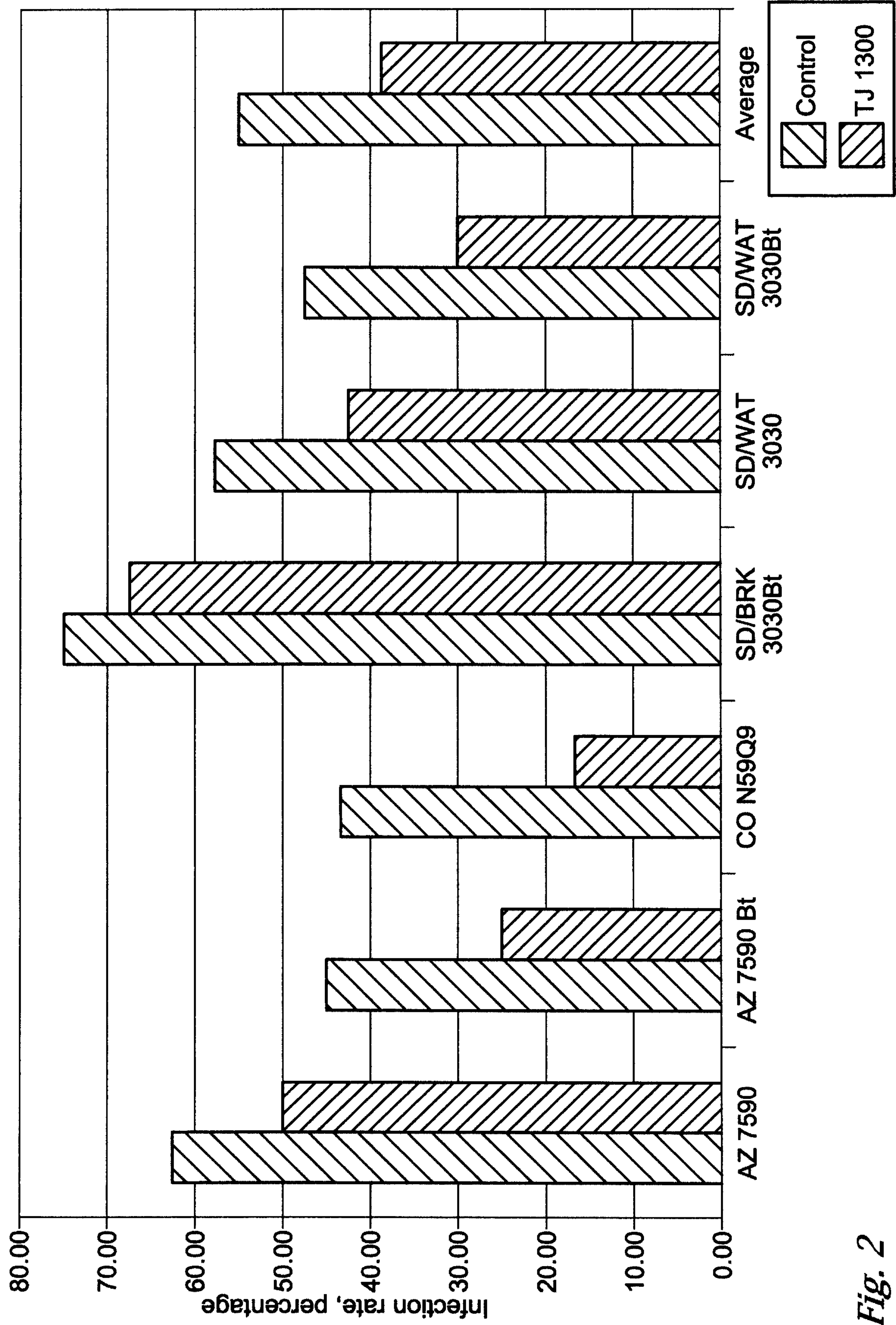


Fig. 2