

Biological Control of Fusarium Head Blight with *Bacillus subtilis* TrigoCor 1448

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Introduction

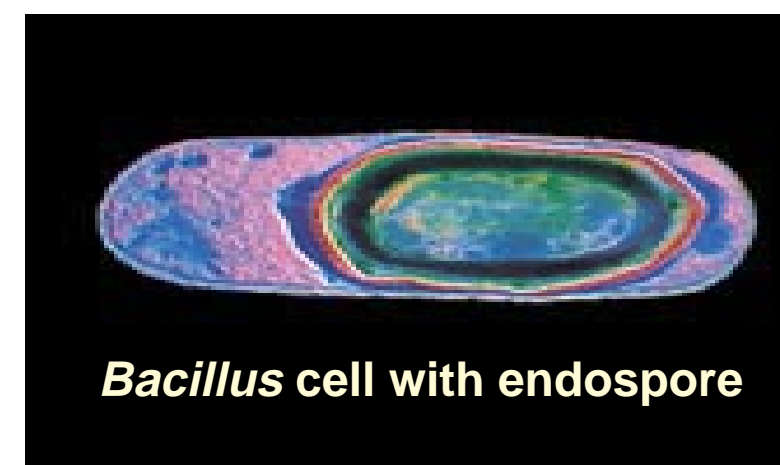
Efforts are being made to provide safe, affordable and efficacious biological protectants for the integrated management of Fusarium head blight (FHB) of wheat and barley caused by *Fusarium graminearum*. The reduction of DON (deoxynivalenol) contamination of the harvested grain remains of critical importance in their management of this disease.

We have directed much of our research efforts with bioprotectants to TrigoCor 1448, a spore-forming bacterial isolate originating from the biocontrol program of Dr. Luz at EMBRAPA-TRIGO, Brazil.



Identity of the bacterial isolate

TrigoCor 1448 has been identified as *Bacillus subtilis* with 99% confidence based on the sequence of a 500 bp segment of the 16S rRNA using NCBI BLAST search of the GeneBank's nucleotide database. 529 out of 532 base pairs of the 16S ribosomal RNA gene of 1448 matched those of an industrial *Bacillus subtilis* strain (TB11). There are several other close matches with other *B. subtilis* strains and with strains only identified as *Bacillus* sp. Similarity with other bacterial species drops off sharply outside of this group.



Bacillus cell with endospore

Laboratory and Growth Chamber Experiments

Antibiosis assay

Ten plates of nutrient agar were inoculated with TrigoCor 1448. Bacteria were transferred to the surface of the agar in a circular imprint using the rim of a sterile funnel. Twenty-four hours later, a 4 mm diameter disk cut from a culture of *G.zeae* was placed in the center of the ring of bacteria. Ten additional plates of nutrient agar inoculated with the fungus alone served as the control. When the edges of the fungal colony in the control treatment plates had expanded to the edge of the plate at 6 days after fungal inoculation, measurements were taken on the radius of the fungal colonies on all plates. Fungal growth was reported as the average of perpendicular measurements of the colony radius from the outer edge of the agar disk.

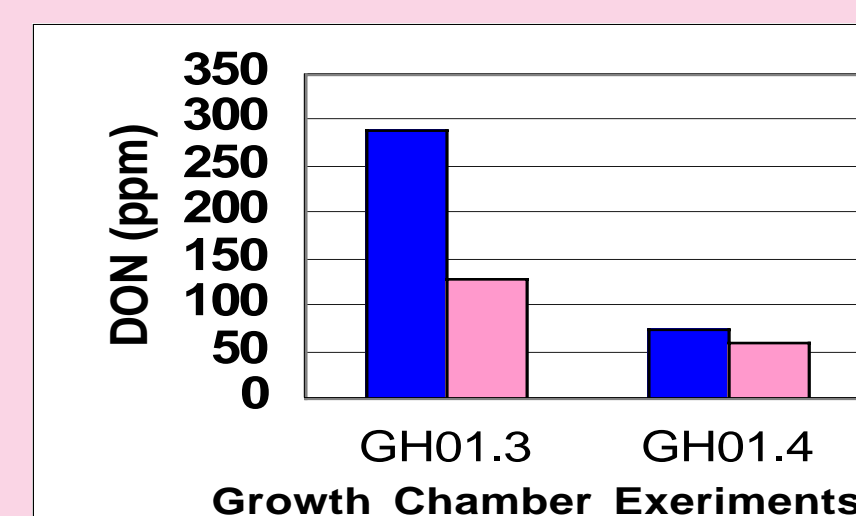
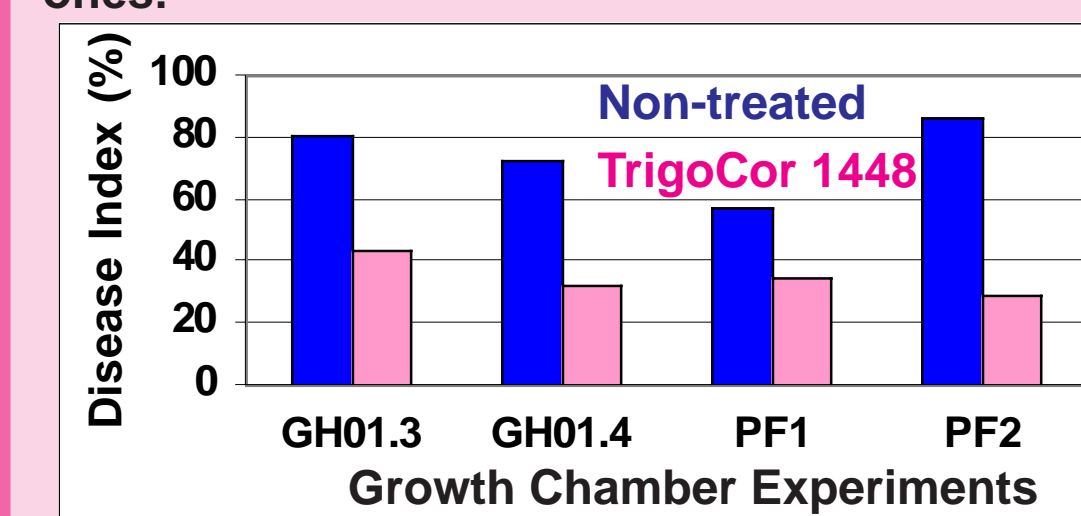


Radial growth of *G.zeae* on nutrient agar in the presence of TrigoCor 1448 was significantly less than its growth in the absence of the bacteria (control). From this it is clear that one or more diffusible antibiotics were being produced by TrigoCor 1448. Collaborative work is in progress to identify the antibiotics produced by this isolate in culture.

Treatment	Radius (cm)
Control	3.7
TrigoCor 1448	0.51

Growth chamber experiments

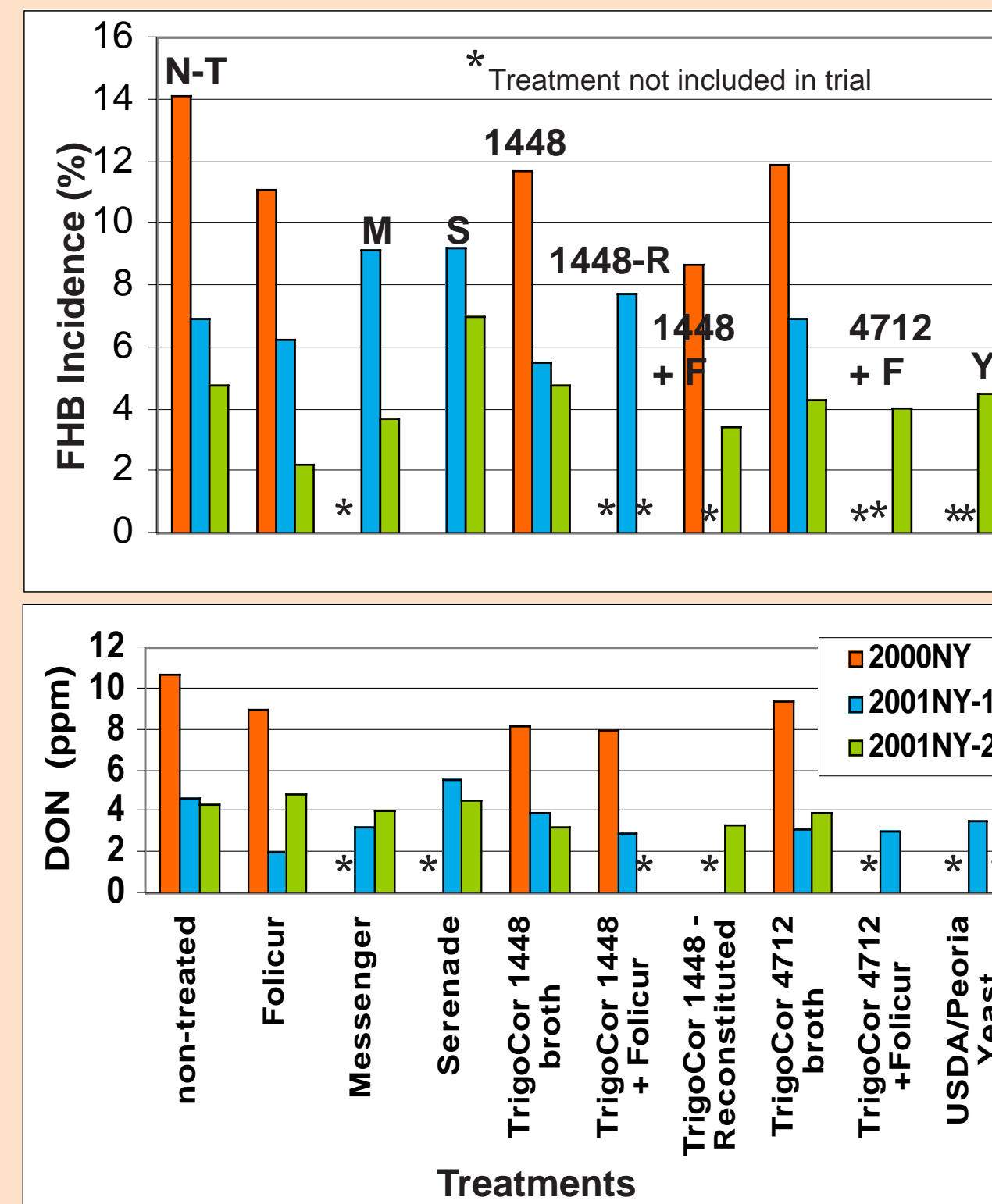
Four experiments were conducted in growth chambers: GH01.3 and GH01.4, at Cornell Univ. and PF1 and PF2, in Passo Fundo, Brazil. All plants were challenge inoculated with *G. zeae*. There were 20 and 4 repetitions per treatment respectively in the Cornell and Brazilian experiments. In all four experiments, there was a significant difference in disease index (disease incidence x severity) between the TrigoCor 1448-treated plants and the control. DON contamination was determined for the harvested grain in two experiments. While DON levels were extremely high, the results clearly show a highly significant reduction in DON contamination in the TrigoCor-treated plants compared to the non-treated ones.



2000 and 2001 Field Trials - NY

The bioprotectants TrigoCor 1448 and TrigoCor 4712 were included in field trials at Ithaca, NY in 2000 and at the Musgrave Research Center, Aurora, NY in 2000 and 2001. Our isolates, grown for 5 days with constant agitation in nutrient broth, had a cell concentration of 2-4 x 10⁸ cfu/ml and were applied undiluted (Aurora 2001) or diluted (in the other trials). The USDA/Peoria Yeast was applied according to directions. The synthetic fungicide, Follicur, was included for comparison.

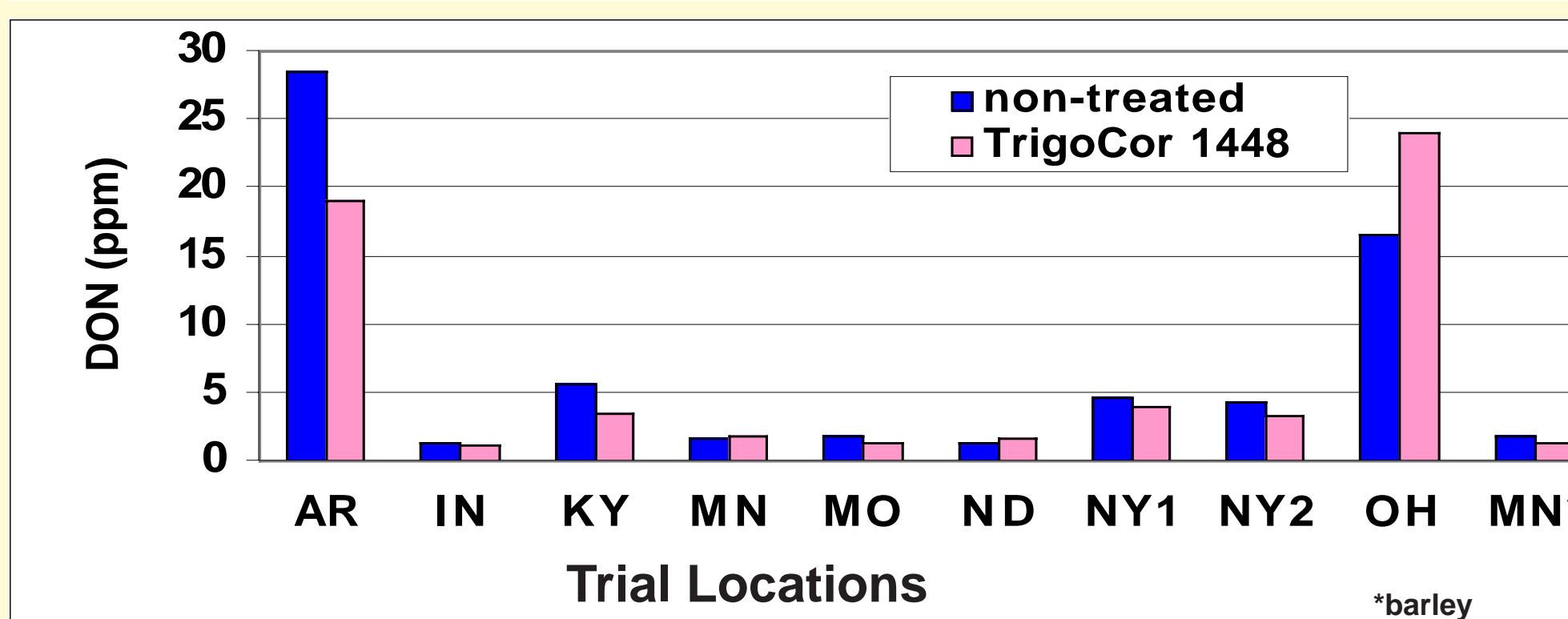
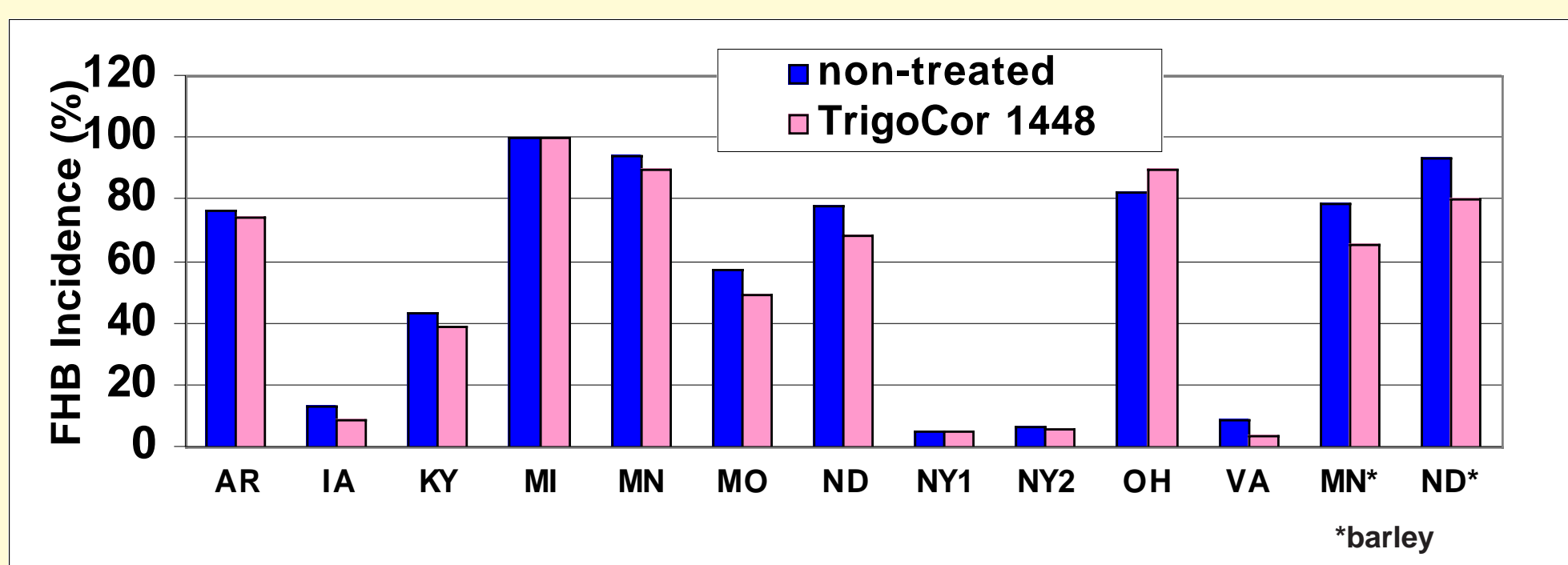
TrigoCor 1448 reduced FHB incidence and DON levels in both years. Wheat treated with TrigoCor 1448 combined with Follicur had the lowest amount of DON of all treatments in the 2000 trial. In 2001, the bioprotectant-fungicide treatment reduced DON levels below that of plants treated with any of the bioprotectants alone.



2001 National Uniform Fungicide/Biological Trials

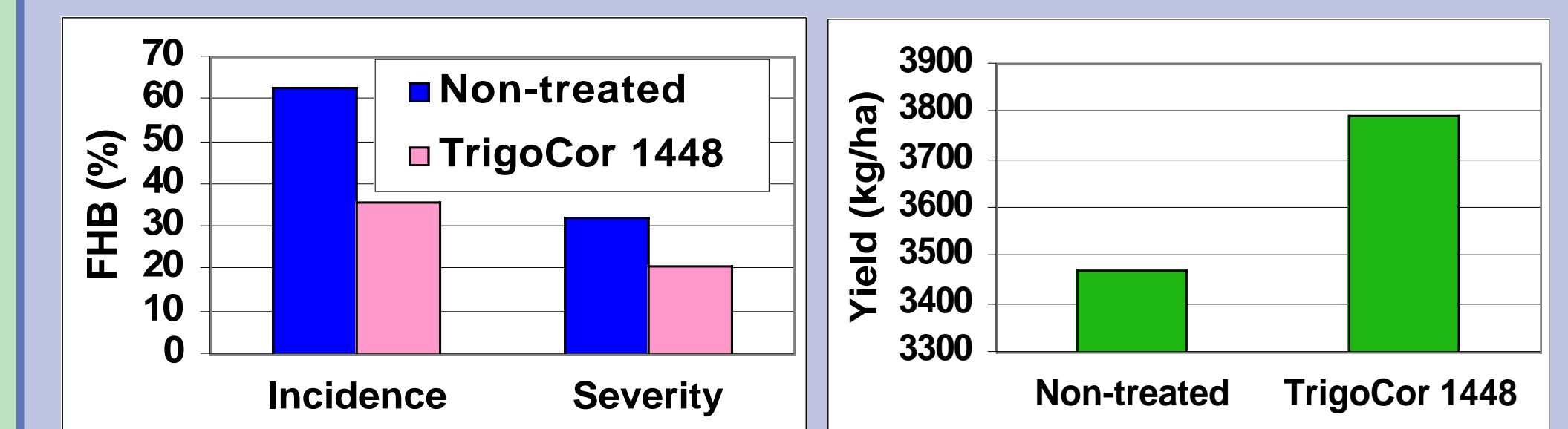
A core set of treatments including the bioprotectant TrigoCor 1448 were tested at 15 sites in 13 states. A culture of the bioprotectant was sent to each collaborator along with instructions and dry ingredients to make sufficient nutrient broth culture for field application.

At 11 out of 15 sites, the incidence of FHB was reduced by treatment with TrigoCor 1448. Ohio was the only site with substantial FHB in which this trend was reversed. Of 10 field trials where non-treated grain was contaminated with > 0.5 ppm DON, 7 showed a decrease in DON or an increase of less than 0.5 ppm in response to TrigoCor 1448 application. Under severe epidemics at Arkansas, and Kentucky TrigoCor 1448 reduced DON by 33% and 39% respectively.



Field Trial - Passo Fundo, RS, Brazil

TrigoCor 1448 was included in a 1999 field trial to evaluate control of FHB by bioprotectants at Passo Fundo, RS, Brasil. The bacteria were applied as a suspension made from a powdered formulation produced by EMBRAPA. This experiment relied upon natural inoculum and precipitation. Spikes were rated for disease 15 days after anthesis. TrigoCor 1448 significantly reduced disease incidence and severity compared to non-treated plants. In contrast to most North American trials, yield was significantly higher in the TrigoCor 1448-treated plants.



Summary

- When grown on nutrient agar, TrigoCor 1448 produces one or more diffusible antibiotics that inhibit the growth of *G. zeae*.
- In growth chamber experiments, treatment of the spikes with TrigoCor 1448 before challenge inoculation with *G. zeae* significantly reduced Fusarium head blight and DON contamination of the grain (up to 55%).
- In NY field trials in 2000 and 2001, treatment of flowering spikes with TrigoCor 1448 significantly reduced FHB and DON contamination.
- In the NY 2000 trial, wheat treated with TrigoCor 1448 combined with Follicur had the lowest amount of DON of all treatments. In 2001, the same treatment reduced DON levels below that of plants treated with any of the bioprotectants alone. These promising results suggest a strong potential for use of bioprotectants in an integrative approach to FHB control.
- In national field trials, TrigoCor 1448 reduced the incidence of FHB compared to non-treated plants at 11 out of 15 sites.
- In national field trials, DON contamination was reduced by TrigoCor 1448 at 7 out of 10 sites where DON > 0.5 ppm was reported. In severe FHB epidemics in Arkansas and Kentucky trials, TrigoCor 1448 reduced DON by 33% and 39%, respectively.
- In a field trial in Brazil in a natural FHB epidemic, TrigoCor 1448 reduced incidence and severity by 29% and 34%, respectively. In contrast to many North American trials, the yield of the TrigoCor 1448-treatment in Brazil was significantly higher than non-treated plants.
- Ongoing efforts are focussed on gaining a better understanding of the FHB biocontrol process and improving the efficacy of bioprotectants alone or in combination with chemical fungicides.



Acknowledgments

We wish to thank all of the regional collaborators in the 2001 Uniform Fungicide Trial who included TrigoCor 1448 as a core treatment and provided us with results: Gene Milus/ AR, Greg Shaner/ IN, Gary Munkvold/ IA, Don Hershman/ KY, Arv Grybaukas/ MD, Par Hart/ MI, Hala Toubia-Rahme/ MN, Laura Sweets/ MO, Pat Lipps/ OH, Marcia McMullen/ ND, Marty Draper/ SD and Erik Stromberg/ VI. We also extend our thanks to the laboratory of Pat Hart at Michigan State Univ., for the determination of DON contamination of seed harvested from our glasshouse and field trials.