BARD

FINAL REPORT

PROJECT NO. US-2880-97

**Enhancing Crop Yields through Colonization of the
Rhizosphere with Beneficial Microbes**

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**BARD Final Scientific Report
Cover Page**

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Project Title: Enhancing Crop Yields through Colonization of the Rhizosphere with Beneficial Microbes

Investigators

Principal Investigator (PI): Gary E. Harman

Co-Principal Investigator (Co-PI): Ilan Chet

Collaborating Investigators: None

Institutions

Cornell University

Hebrew Univ. of Jerusalem

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Maize, cucumber, *Trichoderma harzianum*, nitrogen fertilizer use efficiency, enhanced nutrient uptake, induced resistance

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$138,000

US: \$147,000

Total: \$285,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)		3	3	6
Submitted, in review, in preparation			1	1*
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

*Additional papers will result from this research; the research is being completed.

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Bruno Donzelli, Cornell University, SS#101-86-2037

Iris Yedidia, Hebrew University of Jerusalem

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	2	1	4
Longer Visits (Sabbaticals)				

Telephone and email conversations occur frequently.

Description of Cooperation:

The research conducted in this proposal was designed to be complementary. We had good evidence that our original views of mechanisms of beneficial microbes, which had focused on direct effects on deleterious microbes, were too limited. Instead, in this research, the beneficial microbes were shown to be opportunistic plant symbionts with many distinct and different effects directly on plants. The US and Israeli groups focused on different effects and showed just how diverse and useful the symbionts can be to agriculture. The expectation now is that future cooperative research will be more tightly integrated and will provide comprehensive and detailed universal basic and applied information.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted		1		1
Issued (allowed)				
Licensed		1		1

ABSTRACT

At the start of this project, fungi in the genus *Trichoderma* were known to be potent biocontrol agents, and their primary mechanism was considered to be via direct effects upon the target fungi. Due in large part to the efforts of the two PIs, we now know that this view is far too limited; while *Trichoderma* spp. do indeed have direct effects on pathogenic fungi, they have very far reaching effects directly upon plants. Indeed, these fungi must be considered as opportunistic plant symbionts; they provide a number of benefits to plants and themselves are favored by large numbers of healthy roots. Research under this BARD grant has demonstrated that

- These fungi induce resistance mechanisms in plants.
- They increase root development and depth of rooting; *Bradyrhizobium* enhances this effect in soybean.
- They enhance uptake of plant nutrients.
- They have abilities to solubilize nutrients, such as oxidized metals and insoluble phosphorus compounds by a variety of different mechanisms and biochemicals.

This is a marked expansion of our knowledge of the abilities of these organisms. This knowledge has direct implications for understanding of basic plant responses and abilities, and already is being used to improve plant productivity and reduce pollution of the environment.

ACHIEVEMENTS

This research has achieved a number of conceptual and applied breakthroughs. These will be considered by topic below. Many of these have direct benefit to agriculture and are commercially valuable; this value will be discussed in the last part of this section.

Induced resistance

We considered, until about the start of this research project, that pathogenic fungi were controlled by *Trichoderma* spp. primarily by direct parasitism or by antibiotic production. In large part as a consequence of research done in Israel, we now know that these fungi induce natural plant defense mechanisms and that this is an important means of disease control. Among the recent findings with the Israeli strain T-203 of *T. harzianum*:

- Abundant hyphae grow over the entire surface of cucumber roots and fungal distribution is similar on all parts of the root system (5,6).
- Coiling of the strain over root hairs is similar to that observed in mycoparasitism of other fungi, suggesting similarities in the mechanisms of rhizosphere competence and mycoparasitism (5).
- Root colonization by the strain results in penetration of the epidermis and cortex of roots by hyphae of the fungus (5).
- Root colonization by T-203 has resulted in induction of the systemic resistance factors produced in both leaves and roots. The types of resistance factors induced were similar, but not identical, to that of a 2,6-dichloroisonicotinic acid, which induces systemic acquired resistance (6).
- Root colonization by T-203 induces resistance to the bacterial pathogen *Pseudomonas syringae* pv. *lachrymans* in cucumber foliage. This resistance is associated by production of several antifungal compounds. One of these, named prosopinine, was chemically characterized for the first time from a cucurbit and is a novel compound first discovered in a plant in the family Mimosaceae (7).

Enhanced Root Development

T. harzianum strains enhance root development on many crops. These abilities have been examined in detail at Cornell, primarily with maize in the field. A summary of these findings follows:

- Strain T-22 is strongly rhizosphere competent and colonizes existing and newly formed roots, thus providing long-lasting benefits to crop plants.
- It enhances root growth and development; roots of mature (2 m tall) maize may be twice as numerous 0.3 to 1 meter below the soil surface on plants grown from T22-treated seeds as compared to those from nontreated seeds. This deep rooting provides a number of substantial benefits, including:
 - _ It substantially enhances nitrogen fertilizer use efficiency; field research at Cornell indicates that nitrogen fertilizer in maize can be reduced 40-50% with no yield penalty (3). These results are being widely tested in the maize belt of the midwestern USA. This discovery should lead to amelioration of the severe problem of excessive nitrate pollution of groundwater from maize production.
 - _ Deeper roots increase tolerance to drought and probably also to other abiotic stresses such as compacted soils (3).
- Other recent discoveries indicate that T22 improves soybean root architecture and that the addition of *Bradyrhizobium* improves root structure and development even more (recent unpublished results at Cornell).

Enhanced Nutrient Uptake and Solubilization

T. harzianum strains also affect nutrient status of plants. T-203 in hydroponic culture increased uptake of Cu, P, Fe, Zn, Mn and Na into cucumbers. Since all nutrients were in solution, this ability no doubt results from an improved active uptake capacity of the roots in the presence of the organism (6). In addition, strain T-22 has the ability to solubilize a number of insoluble plant nutrients, including Cu^{+2} , Fe^{+3} , Mn^{+4} , Zn metal and rock phosphate (1).

Gene Control

The mechanisms and processes by which genes of importance are regulated in these fungi is critical to understanding the ecological and physiological processes by which *Trichoderma* spp. improve plant productivity and control disease. Several genes are strongly regulated by both carbon and nitrogen availability (2), which probably is important in various processes and may be important in their ability to enhance plant nutrient status.

Economic and Environmental Benefits

These findings have the potential for substantial improvements to the US and Israeli economies both through improvement of agricultural productivity, reducing environmental costs and through providing new goods and services. Some of these advantages follow:

- T-22 conservatively is expected to replace at least 25% of the total N fertilizer on maize, if applied over 10 to 20% of the maize acreage of the USA, farmers would save \$20 to 100 million annually depending upon the current price of N fertilizer.
- We expect, but have not yet proven, that this reduction in N fertilizer will substantially reduce nitrate pollution of ground and surface water; the US EPA estimates costs of this pollution (from all sources) to be in excess of \$1,000,000,000.
- Until very recently, *Trichoderma* spp. were expected only to control plant pathogenic fungi at the site where they were applied or colonized. Their recently discovered abilities to provide systemic induced resistance in plants broadens the potential range of pathogens controlled to bacteria, viruses and nematodes, as well as fungi. This coupled with long-term colonization and benefits should provide important new tools for highly economical pest control with minimal environmental pollution.

PUBLICATION LIST

1. Altomare, C., Norvell, W. A., Björkman, T. & Harman, G. E. 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol., 65: 2926-2933.
2. Donzelli, B. G. G. and Harman, G. E. 2001. Interaction of ammonium, glucose and chitin regulates the expression of cell wall-degrading enzymes in *Trichoderma atroviride* strain P1. Appl. Environ. Microbiol. 67:5463-5647.
3. Harman, G. E. 2000. The dogmas and myths of biocontrol. Changes in perceptions based on research with *Trichoderma harzianum* T-22. Plant Dis., 84: 377-393.
4. Yedidia, I., Benhamou, N. & Chet, I. 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied and Environmental Microbiology, 65(3): 1061-1070.
5. Yedidia, I., Benhamou, N., Kapulnik, Y. & Chet, I. 2000. Induction of accumulation of PR proteins activity during early stages of roots colonized by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiol. Biochem., 38: 1-11.
6. Yedidia, I., Srivasta, A. K., Kapulnik, Y. & Chet, I. 2001. Effect of *Trichoderma harzianum* on uptake of microelements and increased growth of cucumber plants. Plant Soil, 235: 235-242.
7. Yedidia, I., Kerem, Z., Benhamou, N., Kapulnik, Y., Chet, I. Induced systemic resistance against *Pseudomonas syringae* pv. *lacydiana* elicited by *Trichoderma harzianum* includes the synthesis of prosopinine a novel phytoalexin in cucumber. Ms. in preparation.