Trichoderma: A Potent Fungus as Biological Control Agent
Case Study 14

Research goal	To control pathogenic fungi in field crops through root colonization by *Trichoderma*, an endophytic fungus. At a later stage, the role of *Trichoderma* in inducing multiple benefits to plants, such as root growth promotion, resistance to abiotic stress and increased nitrogen use efficacy, was also examined.
Beneficiaries	Field and Greenhouse Farmers; Ornamentals, vegetables and row crops
Activities conducted in order to achieve the objectives	Protoplast fusion was conducted to obtain highly rhizosphere competent *Trichoderma* strains that are effective against pathogens and promote plant growth. Isolation of antifungal enzymes Study of gene expression changes leading to growth promotion
Funding	BARD awards: $2 Million, Industry: $13 Million.
Publications	50 journal publications, 16 of them in the top IF quartile, 18 with more than 100 citations and 2 with over 700 citations
Students involved	7 post-doctorate researchers were involved in the BARD research projects. Currently, four of them have academic positions; 2 in Israel, 1 in the US and 1 in Taiwan. Two continued to positions in biotechnology industry, 1 in Israel and 1 in the US.
Stakeholders' collaboration	Farmers
Environmental impact	Reduction is use of chemical fungicides Reduction in atmospheric carbon as a result of increased sequestration of atmospheric CO_2, due to both enhanced photosynthesis and elevation of soil organic matter.
Social impact	Worker safety (as a result of the reduction in chemical applications), Strengthening of small stakeholders in developing countries
Commercial engagement	Eastman Kodak Company; Bioworks; Advanced Biological Marketing
Patents	11 Patents issued
Practical agricultural applications	*Trichoderma* application as a soil or seed treatment to protect plants against a large spectrum of diverse pathogens under varied agricultural conditions, and to act as a plant growth promoter. Products developed as a direct outcome of the research are sold globally by two US based companies; one focused on greenhouse crops and one on row crops.
Economic impact	Net present value of the BARD's investment is $647 million, thereof $190 million already attained The Internal rate of return is 17% Benefit cost ratio is 85, thereof 26 already attained

Green- Academic information; Yellow - Social and environmental information; Blue - Economic information

Objective:

Trichoderma, an endophytic fungus has been known from the 1930's to act as a control agent against soil-borne plant pathogens through plant root colonization. The initial objectives of the research were to elucidate the mechanisms by which seeds treated with *Trichoderma* induced plant resistance against soil-borne pathogens, and to use this knowledge to enhance the activity and efficacy of *Trichoderma* applications. As the systemic benefits of *Trichoderma* treatment emerged, the research evolved into understanding the fungal-induced mechanisms by which plants enhanced their productivity and resilience.

2 Research Activities

Between 1981-2007 eight BARD awards were granted to Ralph Baker (Colorado St. University), Gary Harman (Cornell University) and Ilan Chet (Hebrew University). See Appendix A for full details of the awards.

The collaboration between the researchers began during concurrent sabbaticals in 1980 of Ilan Chet and Gary Harman in Ralph Baker's laboratory, who at the time was conducting research on the biocontrol action of *Trichoderma hamatum.* In the first BARD grant the researchers discovered that the biocontrol activity of *T. hamatum* was dependent on soil type and composition, and they then conducted protoplast fusion (a method of obtaining asexual hybrids of these nonsexual fungi) between numerous different trichoderma strains and species in an effort to obtain highly rhizosphere competent strains that were able to compete with bacteria in the soil around the vicinity of the seed. Thousands of resulting progeny strains were then tested for pathogen biocontrol. One of the strains that was effective against pathogens in varied environments, and was also shown to improve the plants' root development was the fusion progeny of two *T. harzianum* strains, now widely known as T-22. Strain T-22 was the focus of the greater part of the follow up studies by the researchers.

Early findings showed that T-22 colonization was obtained over the entire root surface when delivered by a variety of methods (e.g. seed treatment, soil-surface and in-furrow granules, drench), that colonization generated season-long benefits and that plant growth was enhanced, i.e. the effects of the T-22 were systemic to the plant.

Later research identified specific enzymes (chitinolytic and glucanolytic) generated by *Trichoderma* that were strongly antifungal and synergistic with each other. The genes were isolated and found to markedly induce resistance in plants when introduced as transgenes.

Research on gene and protein expression in crop plants demonstrated that most of the beneficial effects of *Trichoderma* on plants were due to systemic changes in the plants' gene expression, leading to enhanced productivity and resilience.

Gene expression changes were demonstrated to lead to enhanced photosynthesis and to produce proteins which detoxify reactive oxygen species (ROS) created under biotic and abiotic stress conditions (e.g. drought and salinity).

The BARD funding enabled to conduct, concurrently, both basic and applied research.

3 Academic Impact

3.1 Publications

50 peer-reviewed journal publications have been published based on the research from the 8 BARD awards. Of these, 16 were in the top IF quartile, 18 have been cited more than 100 times, and 2 were cited more than 700 times.

3.2 Capacity Building

Seven post-doctorate researchers were involved in the BARD research projects. Currently, four of them have academic positions; 2 in Israel, 1 in the US and 1 in Taiwan. Two continued to positions in biotechnology industry, 1 in Israel and 1 in the US.

3.3 Stakeholder's Collaboration

Overall, between the early 1990's and 2002, visiting scientists from within the US, Switzerland, Italy, Spain, Austria, Holland, Norway, Denmark, China, South Korea, and Nepal spent both short and extended time periods visiting and working in the Harman lab at Cornell. The scientists collaborated on the research on chitinolytic and glucanolytic enzymes, the changes in gene regulation of plants induced by *Trichoderma* and improved methods of fermentation and production of *Trichoderma* spores.

G. Harman co-founded Bioworks for the development of commercial systems for applications of *Trichoderma* (see section 4) and later was the CSO of Advanced Biological Marketing from the time of its establishment (see section 4). The commercial development aspects of these companies were interwoven with the research at Cornell. Information was obtained on the commercial side that was useful for work at Cornell, and *vice versa*. The two sides of this development partnership were synergistic, and progress was more rapid with this hybrid R&D approach than it would have been otherwise.

4 Commercial Engagement

Eastman Kodak Company

The rights to the T-22 patent was licensed by Cornell to the Eastman Kodak Company who had established a new agricultural biotechnology division. In 1990 Kodak terminated their efforts to develop a biotech division (and commercialization of *Trichoderma* applications) due to external reasons. However, they had obtained registration for the *Trichoderma* T-22 strain, one of the very first US Environmental Protection Agency registrations for a microbial pesticide. This greatly facilitated the next stage of commercial development. The registration of T22 was gifted to the Cornell Research Foundation.

TGT Inc/BioWorks

The development of the *Trichoderma* biocontrol technology continued with the establishment of TGT Inc. co-founded by G. Harman (Chief Scientific Officer). T. Stasz, his Ph.D. and post-doctoral researcher on the BARD grants (VP Scientific Officer) and a third founder as CEO. TGT evolved into BioWorks (1993) and received the rights from the Cornell Research Foundation to use the patented strain.

Bioworks developed methods to increase stability of the conidia following the scale-up fermentation process, a difficulty encountered by Kodak in their fermentation facilities. Today, the commercial success worldwide of products containing *Trichoderma* is attributed to the large volume of viable propagules that can be produced rapidly and readily on numerous substrates at a low cost in diverse industrial fermentation systems. In the mid '90s, G. Harman left his official positions (CSO, Interim CEO) at Bioworks to continue solely at Cornell as a full-time university faculty member.

Advanced Biological Marketing (ABM)

In 2000, a new company, Advanced Biological Marketing (ABM), was established, and advanced the use of the developed *Trichoderma* technologies for field crops. ABM licensed the use of T-22 for row-crop seed treatment with a focus on seed treatments on soy, corn, wheat and cotton. The company also conducted R&D through grants to G. Harman at Cornell as a contractor until his retirement (2000-2012), after which he became the CSO of ABM until 2017.

In 2006, using the protoplast fusion progeny collection (developed as part of the BARD supported research), 3 novel strains of *Trichoderma viride* were identified that gave consistently good yield results with corn and other crops. To date, the *Trichoderma* strains comprise around 50% of the company's revenues. The strains were patented by Cornell and are exclusively licensed to ABM for worldwide rights during the patent's lifetime license (expiration due in 2030). These new *Trichoderma* strains became the mainstay of the ABM products together with *Rhizobia* formulations used for legume seed treatments. The two products are also combined for some crops such as soybeans.

5 Patents

1. *Purified chitinases and uses thereof*, Gary Harman, M. Broadway, A. Tronsmo, and M. Lorito; US5,173,419; Issued: 1992-12-22 to Cornell Research Foundation
2. *Fused Biocontrol Agents*, Gary E. Harman, Thomas E. Stasz, Norman F. Weeden, US5,260,213, Issued 1993-09-09 to Cornell Research Foundation,

3. *Method of increasing the percentage of viable dried spores of a fungus,* Gary Harman, X. Jin., T. E. Stasz, G. P. Peruzzotti, A. C. Leopold, and A. G. Taylor; US5,288,634; Issued: 1994-04-22 to Cornell Research Foundation
4. *Fungicidal compositions comprising chitinase and enterobacter cloacae, and a method for stimulation proliferation of E. Cloacase,* Gary E. Harman, Matteo Lorito, Christopher K. Hayes; US5360608A, Issued: 1994-11-01 to Cornell Research Foundation
5. *Gene encoding for endochitinase*, Gary E. Harman, Arne Tronsmo, Christopher K. Hayes, Matteo Lorito, US5378821A, Issued: 1995-1-03 to Cornell Research Foundation
6. *Antifungal synergistic combination of enzyme fungicide and non-enzymatic fungicide and use thereof*; Harman, G. E. Lorito, M., Di Pietro, A., and Hayes, C. K. Issued: 1995-7-18 to Cornell Research Foundation
7. *A N-acetyl-ß-glucosaminidase isolated from Trichoderma harzianum;* Harman, G. E., Hayes, C. K., Lorito, M., and Di Pietro, US5474926A, Issued: 1995-12-12 to Cornell Research Foundation
8. *Gene encoding endochitinase,* Gary E. Harman, Arne Tronsmo, Christopher K. Hayes, Matteo Lorito and Sonja Klemsdahl; US6020540A, Issued: 2000-2-01 to Cornell Research Foundation
9. *Purified chitinases and use thereof*; Harman, G. E., Broadway, R. M., Tronsmo, A., Lorito, M., Hayes, C. K. and DiPietro, US6251390B1, Issued: 2001-6-26 to Cornell Research Foundation
10. *Combinations of fungal cell wall degrading enzyme and fungal cell membrane affecting compound*; Harman, G. E., Lorito, M., Di Pietro, A., Hayes, C. K., Scala, F. and Kubicek, C. P, US6512166B1, Issued: 2003-1-28 to Cornell Research Foundation
11. *Trichoderma strains that induce resistance to plant diseases and/or increase plant growth,* Gary Harman; US8877481B2; Issued: 2014-11-04 to Cornell University

6 Practical Agricultural Applications

The results of this project have led to the development of biocontrol products used to protect plants against a large spectrum of diverse pathogens under numerous agricultural conditions, and to act as a plant growth promoter *via* enhanced root growth, increased seedling vigor, and improved nutrient and water efficiency. *Trichoderma* is applied as soil treatments against both soil-borne and foliar-borne pathogens. *Trichoderma* is broadly resistant to fungicides, permitting the use of both the biological root colonizing agent and chemical seed protectants.

T-22 was among the first *Trichoderma* strains to be commercialized of the disease-antagonistic fungus *Trichoderma harzianum*. This pioneering strain, T22, has supported the development of these fungi for agricultural use around the world in a variety of field, greenhouse, nursery, horticultural, fruit, and ornamental crops. The T22 based products marketed by BioWorks Inc. are a mainstay of their product line, in combination with the *Trichoderma virens* strain G-41 developed and patented by the company and which is especially effective against *Phytophthora* root disease.

Successful sales of Bioworks products began in 1993, when T22 based formulas were applied to ornamentals grown in greenhouses, providing defense against the dominant root disease, *Pythium.* To date, sales of the range of products are split equally between application to both greenhouse (mostly ornamentals) and outdoor crops (vegetables).

Bioworks *Trichoderma* based products gained a significant foothold in agriculture in about 1998, and since then sales have increased about 20% per year[1]. Sales are global, with the bulk being to North America: USA, Canada and Mexico.

In 2009, ABM began to market different mixtures of proprietary strains of trichoderma as root and soil inoculants and biostimulators for wheat, corn, cotton and vegetables and to combine the strains with Rhizobia for legume seed protection. The ABM products provide both enhanced yields, induce resistance to abiotic stresses such as drought and salt and especially for potatoes, yams, tomatoes, and onions, improve the quality of the crop. These products have become widely used in the US and around the world and are marketed in 14 countries including: Philippines, South Korea, Vietnam, Indonesia and a number of African countries. ABM products are sold as bio-stimulants for soy, corn, cotton, wheat, rice, vegetables and additional crops as described on their site[2] (in descending order of revenues from sales). The average increased yields of crops treated with ABM *Trichoderma* are shown in the Table 1 (see section 9.2). Additionally, for crops such as potatoes, yams, tomatoes, and onions, the quality of the crop is greatly improved.

At the time of entrance into the marketplace, the novel *Trichoderma* inoculants were a disruptive technology, and adoption was slow. Today, there are more than 250 *Trichoderma* based products available worldwide. They are marketed as bio-pesticides, biofertilizers, growth enhancers and stimulants of natural resistance. The largest distribution of *Trichoderma* bioproducts is found in Asia (predominantly India), succeeded by Europe, South-Central America and North America. The living fungal spores (active substance) are incorporated in various formulations for applications as foliar sprays, pre-planting applications to seed or propagation material, post-pruning treatments, incorporation in the soil during seeding or transplant, watering by irrigation or applied as a root drench or dip.

[1] http://www.hort.cornell.edu/department/faculty/harman/pubs/changing_models.pdf
[2] https://www.abm1st.com/innovation/iget-technology/

7 Environmental impact

The Bioworks products are primarily used as biocontrol of root disease (*Pythium, Phytophthora* and more*).* The products replace application of chemical fungicides, some of which are now less effective due to development of pathogen resistance. The products adhere to strict organic requirements and also provide a response to consumer, retailers and legislative demands for reduced application of chemical fungicides.

The use of the *Trichoderma* products as growth promoter, as in the ABM product lines, can contribute to a reduction in atmospheric carbon as a result of increased sequestration of atmospheric CO_2, due to both enhanced photosynthesis and elevation of soil organic matter.

8 Social Impact

Reductions in pesticide use leads to enhanced worker safety and consumer assent for less chemical applications to edible products. In addition, fertilizers are rapidly becoming too expensive for use by smallholders in many parts of the world, and biofertilizers, in their various forms, are considered to be essential components of food security in many poorer countries. Specifically, ABM conducts ongoing work and provision of seed (for free) with small stakeholders farming corn and peanuts in the Congo and additional African countries.

9 Economic Impact

9.1 Investment Cost

BARD contributed~ $2 million in research funds between 1981-2007. Industry investments are estimated at ~ $13 million.[3]

9.2 The Benefits

A farmer's decision to apply *Trichoderma* based products for a number of reasons: a) Pathogen resistance to many chemical fungicides, b) economic reasons c) to enhance worker safety and d) non-chemical, non-GMO.

Economics for Bioworks

For a 10-12-week crop growth cycle, a single application of a Bioworks product replaces a 2.5-fold application of a chemical fungicide (needs to be re-applied every 3-4 weeks).

[3] For T22, between Kodak initially and later BioWorks, approximately $12 million was required for registration, development of a production facility, developing adequate formulations, and an effective marketing system before sales of products based upon this organism really began to grow in *Changing Models for Commercialization and Implementation of Biocontrol in the Developing and the Developed World,* Harman et al., 2010, 94(8):928-939 DOI: 10.1094/PDIS-94-8-0928. From ABM - ~ $1 million dollars for identification of new strains and efficacy field trials.

Annual sales to date are estimated at 500,000 kilograms of products (wettable powder and granules), which is equivalent to ~ $30 million. We assumed gradual development of the sales since the beginning in 1998, and estimated extra-benefit to the farmer, compared to a conventional solution that could be purchased, as 20% of the cost he paid in order to use the materials.

Economics for ABM

The business expectations of ABM over the next five years (2018-2023) is to triple sales worldwide. The U.S. market is approximately 80% of the company's sales. ABM's African business is growing at about 30% a year[4].

We received the company's estimation concerning the benefit to the farmers using its products and about the acreage treated by these products in the US, as shown in Table 1. For example, 2% of the US corn acreage is treated today, which is 1.7 million acres (83×2%). The farmer pays $2 per acre to ABM and his net benefit is $26/acre/year. The total US farmers benefit sums to $50 million in 2018. For the purpose of conservative calculation, we assumed only 75% of this stated benefit. We assumed a gradual development of sales, according to the initial marketing year in each crop.

The two companies, ABM and Bioworks, penetrated a small fraction of the US market, and have recently started their global marketing efforts. Therefore, and taking the growth in the last years, we assume that until 2028 they will gradually double their sales.

Table 1: Increased yields of crops treated with ABM *Trichoderma* products relative to common practice and the annual increased revenue for the US farmer based on 2018 values.

Crop	Initial Marketing Year	Yield Increase: Bushels per Acre	Market Price $ per Bushel	Net Benefit * $ per Acre	US Fractional Treated Area	Total US crop area, Million Acres	Increased Total Benefit to Farmers, $M in 2018
Soy	2009	2.1	8	14	3%	83	37
Corn	2009	8	4	26	2%	83	50
Cotton	2011	47#	1/#	32	1%	10	3
Wheat	2010	5.3	6	28	4%	43	51
Rice	2016	9.5	4.75	41	1%	3	1
Total							142

* After paying $2 per acre to ABM

[4] https://www.croplife.com/crop-inputs/qa-with-abm-on-biostimulants-evolution-distribution/ (from May 2081) and personal communication with CEO of ABM.

9.3 Economic Results

BARD invested in the initial and hence risky part of the project. According to the calculation described in the methodology section we attribute 32% of the benefits to BARD.

- Net present value of the BARD's investment is $647 million, thereof $190 million already attained
- The Internal rate of return is 17%
- Benefit cost ratio is 85, thereof 26 already attained

Almost all the benefit is attributed to the US economy according to the implementation.

Table 2: Main Results, 2018 Million Dollar-Terms

	The Project	BARD	BARD Attained	Thereof to the US	Thereof to Israel	Other Countries
BARD's Share in the Cost	17%					
Share in the Benefit		32%				
Cost	44	8	8	3.8	3.8	
Benefit	2,046	655	198			
Net Present Value	2,002	647	190	618	-4	33
Internal Rate of Return	18%	17%	15%	20%	#NUM!	
Benefit Cost Ratio	46	85	26	161	-1	

9.4 Sensitivity Analysis

The low and high alternative assumptions used in the sensitivity analysis were brought together to estimate results under pessimistic and optimistic scenarios. Table 3 displays the net present value sensitivity results, between the low result: $217 million, to the high result: $1.3 billion.

Table 3: NPV - Sensitivity Analysis, 2018 Million Dollar-Terms

			BARD's Share in the Benefit		
			Low	Central	High
			22%	32%	42%
Change in Benefit	Low	50%	217	320	422
	Central	100%	442	647	852
	High	150%	668	974	1,281

10 Appendix A: BARD Awards

Table 4: Details of the 8 BARD awards

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
US-290 - 81	Control of Plant Diseases with Biological Components of Suppressive Soils				
	Baker, R.D. Chet, I.	Colorado St. U HUJI			1981
US-318 - 81	New Seed Treatments Using Biological Agents and Integrated Techniques to Control Soil-Borne Pathogen				
	Harman, G.E. Chet, I.	Cornell U HUJI			1981
US-679 - 84C	Application of Antagonists to Soil for Disease Control and Induction of Increased Growth Response				
	Baker, R.D. Chet, I.	Colorado St. U HUJI	$180,000	3 year	1984
US-1224-87C	Enhancement of Efficacy of Trichoderma spp. for Biological Control Using Protoplast Fusion				
	Harman, G.E. Chet, I.	Cornell U HUJI	$267,107	3 year	1987
US-1723-90C	Molecular Approaches to Strain Improvement and Determination of the Role of Specific Gene Products in Biocontrol by Trichoderma Spp.				
	Harman, G.E. Chet, I.	Cornell U HUJI	$250,000	3 year	1990
US-2325-94CR	Discovery and Use of Genes and Gene Combinations Coding for Proteins Useful in Biological Control				
	Harman, G.E. Chet, I.	Cornell U HUJI	$255,000	3 year	1994
US-2880-98C	Enhancing Crop Yield through Colonization of the Rhizosphere with Beneficial Microbes				
	Harman, G.E. Chet, I.	Cornell U HUJI	$285,000	3 year	1998
US-3507-04R	Enhancement of plant disease resistance and productivity through use of root symbiotic fungi				
	Harman, G.E. Chet, I. Viterbo, A.	Cornell U HUJI Weizmann Inst.	$310,000	3 year	2004

11 Appendix B: Information providers: Personal communication

G. Harman – PI of BARD grants; Emeritus, Plant Sciences, Cornell University

William Foster – CEO of Bioworks. Inc.

Dan Custis – CEO of ABM. Advance Biological Marketing