August 2019

Biocontrol Agents for pre-and post-harvest
Case Study 12

Research goal	Development of yeast antagonists for biocontrol of pre and post-harvest diseases.
Beneficiaries	Growers, packing houses, exporters, retailers, consumers.
Activities conducted in order to achieve the objectives	Isolation and screening of new biological-control antagonists found on fruit surfaces Application of molecular and biochemical tools to identify the mode of action of the yeast antagonists. Identification of molecular traits and research on additives to enhance the performance of yeast biocontrol agents.
Funding	5 BARD awards: IS-1019-85, US-1374-88C, IS-1940-92C, IS-2310-94, IS-3117-00R, $1,120,000. Additional research funds: $370,000.
Publications	56 journal publications, 16 of them in the top impact factor quartile (Q1), 8 with more than 100 citations.
Students involved	At least 2 post-doctorate and 7 graduate students: Currently positions: 3 are in Academia, of which 2 are in Turkey and 1 is in Italy, 4 are in industry, 1 is a teacher and 1 works at the Israel Ministry of Agriculture Extension Services.
Stakeholders' collaboration	Extensive international cooperation (universities, research institutions, companies). Organisation of international workshops, symposia and conference sessions on postharvest biocontrol.
Environmental impact	Reduction in use and residues of synthetic chemicals used as fungicides to control pre and postharvest fungal pathogens.
Social impact	Reduction in food losses, reduction in fungicide exposure (workers, consumers, environment).
Commercial engagement	FRM, Ecogen, Agro-Green (indirectly Bayer Crop Sciences and Koppert).
Patents	2 patents assigned to USDA, 2 patents assigned to ARO.
Practical agricultural applications	Spray applications to pre- and post-harvest on crops (grapes, strawberries and stone fruit) in the field and in the packing house.
Economic benefit	Net present value of the BARD's investment is $12 million The Internal rate of return is 9%. Benefit cost ratio is 4.

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

1 Objective: Use of Naturally- Occurring Yeasts as Biocontrol Agents

Mounting concerns regarding pesticide residues in the food value chain and detection of fungal strains resistant to fungicides in major crops such as citrus, and other crops such as grapes, strawberries and others, together with regulations to restrict the use of pesticides led the researchers to target naturally-occurring non-antibiotic producing antagonistic microorganisms to control postharvest pathogens of fruits and vegetables as an alternative to the synthetic chemical fungicides.

2 Research Activities

Charles L. Wilson (USDA, ARS), Edo Chalutz (ARO, Min, Agr.) and Wojciech J. Janisiewicz (USDA, ARS) led a BARD award research project (IS-1019-85**)** in 1985 on biological control of post-harvest diseases. Concurrently, a seminal paper by Wilson and Pusey[1] was published on the potential of postharvest biocontrol and their use of a biocontrol agent to control brown rot on peaches and nectarines. The research direction outlined in this paper and conducted in the BARD study was novel, and laid the foundation for ideas and principles that, over the following 30 years, fostered a wealth of research and product development around the world. The expectation was that biocontrol in a controlled post-harvest environment would prove to be more successful than biocontrol agents developed to manage soil and foliar diseases.

This first award was a 1-year feasibility study and due to its successful outcomes, a follow-up 3-year award was granted (US-1374-88C). Michael Wisniewski (USDA, ARS) and Samir Droby (ARO, Min, Agr) joined E. Chalutz in a subsequent BARD award on yeast biocontrol agents (IS-1940-92C) which was then followed by two additional BARD funds (IS-2310-94 and IS-3117-00R) led by S. Droby and M. Wisniewski together with additional researchers. See Appendix A for full details of these awards.

The research conducted as part of these 5 awards identified yeast antagonists effective against a number of pathogens, identified their mode of action, discovered molecular traits and additive and synergistic effects of additional compounds. The studies explored into physical treatments to enhance the efficacy and reliability of the yeast antagonists.

Research stages included:

- Isolation and screening of new biological-control antagonists found on fruit surfaces. Initial targeted pathogens were *penicillium* and *botrytis* rots of citrus and apples. Isolates efficacy were tested *in-vitro* and *in-vivo*, through reapplication to the fruit. At a later stage, a Random Amplification of Polymorphic DNA - Polymerase Chain Reaction (RAPD-PCR) technique was developed to identify

[1] Wilson, C.L., Pusey, P.L., 1985. Potential for biological control of postharvest plant diseases. Plant Dis. 69, 375–378.

epiphytic yeast populations, enabling the screening of a large number of isolates in a relatively short period:

- Application of molecular and biochemical tools to identify and characterize the mode(s) of action of the antagonists.

  Amongst these: the ability of antagonists to adhere to and parasitize pathogen hyphae (demonstrated for the first time), to compete for nutrients, to produce cell-wall-degrading enzymes and to induce resistance.

- Research on chemical and physical pathways to enhance the efficacy of the yeast antagonism. Efficiency was augmented by calcium, UV light and heat treatments to fruit, glycine betaine treatment to yeast and food additives.

  Current joint research of the two principal researchers (also supported by BARD, IS-5040-17) aims to characterize the fruit microbiome for the development of microbial consortia for biocontrol.

3 Academic Impact

3.1 Publications

56 peer-reviewed journal publications were published based on research from the 5 BARD awards. Of these, 16 were published in the top impact factor quartile (Q1) journals. 8 publications were cited more than 100 times.

3.2 Capacity Building

At least 2 post-doctorate and 7 graduate students were involved in the research supported by the 5 BARD awards between 1985 to 2003. Currently 3 are in Academia, of which 2 are in Turkey and 1 is in Italy, 4 are in industry, 1 is a teacher and 1 works at the Israel Ministry of Agriculture Extension Services.

3.3 Stakeholder's Collaboration

Essential to the success of establishing the science of postharvest biocontrol and developing commercial products was the collaborative relationship that was formed between researchers at USDA-ARS (Charles L. Wilson and M. Wisniewski) and at the Volcani Center in Israel (E. Chalutz. and S. Droby). The highly productive collaboration, much of which was enabled through BARD funding, began in 1985 and continues to this day. The most recent research collaboration beginning in 2017 (BARD award IS-5040-17) focuses on the role of fruit microbiome in the biocontrol system, and is facilitated by technological advances that have been made by the use of next-generation-sequencing and meta-omic technologies to characterize the diversity and function of the microbial communities present in and on host fruit tissues.

International cooperation with South Africa, Brazil, Australia, Egypt, Italy, Portugal, Spain, China, Germany, New Zealand, Mauritania, Turkey and Uruguay, which took the form of visiting scientists, graduate students and product-evaluation arrangements.

S. Droby organized 4 BARD supported international workshops and numerous symposia and conference sessions on postharvest biocontrol. He serves as the chair of the Postharvest Pathology committee of the International Society of Plant Pathology (ISPP). S. Droby and M. Wisniewski served as co-chairs of the International Society of Horticultural Sciences (ISHS) working group on postharvest Biological control.

4 Commercial Engagement

The very first commercial biological control product (Aspire™), based on the yeast *Candida oleophila* (Strain I-182), was a direct outcome of the BARD research. It was used for the control of postharvest decay of citrus and pome. Formulation of the product was conducted in Israel with FRM Agricultural Sciences Partnership. The use of this organism was protected by a patent which was licensed to Ecogen Inc. (Langhorne, PA, USA). Ecogen filed for and received EPA registration in 1995 and marketed Aspire™ for use by fruit packing-houses in the United States and Israel from 1995- 2000. After registration, the commercial evaluation of Aspire™ continued in order to better understand how to adapt the use of the product to different packing-houses, environments and to different commodities. Ecogen provided financial support for conducting semi-commercial pilot tests on apples and citrus at USDA-ARS facilities, and at ARO, Israel. Aspire™ was successful as a protective agent but did little to control pre-established and latent infections originating in the orchard. Poor marketing and company management resulted in the disappearance of Aspire™ from the market place[2].

A second biocontrol product to be developed was "Shemer", a formulation that was based on a heat- and osmo-tolerant strain of *M. fructicola* that was isolated from the surface of table grapes. The commercial product was developed through funding from the Israeli company Agro-green, (part of the MINRAV holdings group) and the Chief Scientist of the Ministry of Agriculture, Israel. Shemer was shown to be effective against rots caused by *Botrytis, Penicillium, Rhizopus* and *Aspergillus* on strawberries, grapes and citrus. Shemer is applied to flowers and fruit in strawberry fields several times throughout the growing period. Product sales began in Israel in 2005, and it was used commercially by the organic sweet potato and carrots growers in Israel. The product also addressed the problems of pre-established and/or latent infections.

Agro-green had two commercial products; Shemer and BioNem, a bio-nematicide. The company was bought by Bayer CropScience in 2010 (for €45,000,000). Through funding

[2] Wisniewski et.al., Postharvest Biology and Technology, 122 (2016) 3–10

provided by Bayer CropScience, the Droby group continued research aimed at improving the performance of the yeast formulations.

In 2013 Koppert Biological Systems reached an agreement with Bayer CropScience to take over the exclusive worldwide rights on the marketing, registration and production of the new Shemer product. Koppert paid Bayer $350,000 for the license and will pay royalties to Bayer following sales. ARO were paid 2,000,000 NIS at the time of signing.

Koppert Biological System's began the registration process for use of the *M. fructicola* strain NRRL Y-27328 (Shemer) as a plant protection product within the EU in September 2014[3]. Formal EU approval was granted to the *M. fructicola* strain and an end-product based on the strain for the prevention of post-harvest decay in stone fruit, vines and berries in December 2018[4]. The registration process in the US is ongoing.

4.1 Indirect commercial outcomes:

Leasaffre International (France) developed a commercial biocontrol product based on the same yeast used in Aspire™, *Candida oleophila* (following expiry of the ARO/USDA patent). The product is commercialized by Syngenta, who launched Nexy®, the first biocontrol solution against post-harvest diseases of bananas in 2017. Nexy (containing 81% *C. oleophila*) controls effectively against banana crown rot.

4.2 Patents

Biological control of diseases of harvested agricultural commodities using strains of the yeast candida oleophila, Charles L. Wilson, Michael E. Wisniewski, Edo Chalutz, US5741699A, Granted: 21-04-1998 to USDA.

Inhibiting plant pathogens with an antagonistic microorganism(s), Randy J. McLaughlin, Charles L. Wilson, Edo Chalutz, US5780023A, Granted:14-07-1998 to USDA,

A novel antagonistic yeast useful in controlling spoilage of agricultural produce, methods of use thereof and compositions containing same, Samir Droby, EP1372384B1, Granted: 2-1-2004 to Ministry of Agriculture, Israel

Yeast Metschnikowia fructicola NRRL Y-30752 for inhibiting deleterious microorganisms on plants, Samir Droby, US6994849B2, Granted: 02-07-2006 to Ministry of Agriculture, Israel

[3] EFSA; doi: 10.2903/j.efsa.2017.5084

[4] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.311.01.0020.01.ENG&toc=OJ:L:2018:311:TOC

5 Practical Agricultural Applications

The EU and EPA regulatory permits were requested for the use of *Metschnikowia fructicola* as a spray application to stone fruits (against *Monilinia fructigena* and *Monilia laxa*), strawberries and grapes (against *Botrytis cinerea*)[5],[6].

Benefits of biopesticides can include: a short or zero-day pre-harvest interval, a short restricted-entry interval, and the flexibility for use as a tank mix or rotational partner in an Integrated Pest Management (IPM) program with chemical fungicides.

The biocontrol market in Europe is in continual growth, for example, between 2013 – 2015 the market grew by 20%[7].

6 Economic Impact

6.1 Investment Cost

BARD contributed $1.12 in research funds between 1985 to 2003. Additional funds provided for R&D of products within the Droby group were $370,000.

The industry does not disclose how much has been invested in the latest development stage of the "Shemer" end-product based on *Metschnikowia fructicola.* Documentation of costs for development of a biopesticide are approximated as $3-$7 million[8].

According to a 2011 report, EU regulation costs for biocontrol products, compiled from submissions of earlier years, averaged ~ €1.9 million (split between efficacy tests, toxicology and ecotoxicology studies and other components)[9]. US regulatory costs are less, ~ €0.643 million[10].

6.2 The Benefits

The economic potential of the biocontrol markets has increased significantly during the last decade, but the overall economic potential of single products is small. Due to the nature of BCAs, in particular their host specificity, the market potential is limited. At the time of the Bayer acquisition of Shemer (2009), the accrued future benefits were estimated to be between $15-20M[11].

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[5] DOI: 10.2903/j.efsa.2017.5084; EFSA Journal 2017;15(12):5084

[6] Federal Food, Drug, and Cosmetic Act (FFDCA) Safety Determination for Metschnikowia fructicola strain NRRL Y- 27328

[7] IBMA (International biocontrol manufacturers association_

[8] Estimate taken from:https://marronebioinnovations.com/commercial-challenges-for-bringing-biopesticides-to-market-what-does-it-take-to-get-there/

[9] Regulation of Biological Control Agents (Ed) Ralf-Udo Ehlers, 2011, Springer

[10] Better safe than sorry? The costs of the EU's registration procedure for microbial biocontrol agents, C. Frederiks, M.Sc. thesis, Wageningen, 2017 (Based on EPA, https://www.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-5-registration-fees)

[11] Personal communication from involved party.

A 2015 study evaluated the cost-benefit ratios for an array of biocontrol products to be between 5:1 to >1,000:1[12]. Assuming that the 5:1 ratio is the minimum multiplier anticipated by a company against its investment costs, and based on R&D development costs of $5M (see section 6.1), we estimate the minimum future benefits of the use of Shemer in the EU and USA as $25 M in 2018 terms.

6.3 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 61% of the benefit to BARD.

- Net present value of the BARD's investment is $12 million,
- The Internal rate of return is 9%.
- Benefit cost ratio is 4.

We assume that around 50% of the benefit will occur in the US and the rest will be applied in Europe.

Table 1: Main Results, 2018 Million Dollar-Terms

	The Project	BARD	BARD Attained	Thereof to the US	Thereof to Israel	Rest of the WORLD
BARD's Share in the Cost	46%					
Share in the Benefit		61%				
Cost	8	4	4	1.8	1.8	
Benefit	25	15	15			
Net Present Value	17	12	12	6	-2	8
Internal Rate of Return	10%	9%	9%	9%		
Benefit Cost Ratio	3	4	4	3	-1	

6.4 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 2 displays the net present value sensitivity results, between the low result: $3 million, to the high result: $23 million.

[12] Naranjo et al., Annu. Rev. Entomol. 2015. 60:621–45

Table 2: NPV - Sensitivity Analysis, 2018 Million Dollar-Terms

			BARD's Share in the Benefit		
			Low	Central	High
			51%	61%	71%
Change in Benefit	Low	50%	3	4	5
	Central	100%	9	12	14
	High	150%	15	19	23

7 Appendix A: BARD Awards

Table 3: Details of the 5 BARD awards between 1985 to 2000

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
IS -1019-**85**	Biological Control of Postharvest Fruit Diseases				
	Wilson, C. Chalutz, E. Janisiewicz, W.J.	USDA, ARS. ARO, Min. Ag USDA, ARS	$60,000	1 year	1985
US-1374-**88C**	Biological Control of Postharvest Diseases of Citrus and Deciduous Fruit				
	Wilson, C. Chalutz, E.	USDA, ARS. ARO, Min. Ag	$220,000	3 years	1988
IS-1940-**92C**	Mode of Action of Yeast Biocontrol Agents of Postharvest Diseases of Fruits				
	Chalutz, E. Wisniewski, M. Droby, S. Eilam, Y Chet, I	ARO, Min. Ag USDA, ARS ARO, Min. Ag. Hebrew U Hebrew U	$230,000	3 year	1992
IS-2310-**94**	Ecology, Population Dynamics and Genetic Diversity of Epiphytic Yeast Antagonists of Postharvest Diseases of Fruits				
	Droby, S. Eckert, J.W.	ARO, Min. Ag UC, Riverside ARO, Min. Ag.	$300,000	3 year	1994

	Manulis, S. Mehra, R.K.	UC, Riverside			
IS-3117-**00R**	Enhancement of Postharvest Biocontrol Activity of the Yeast Candida *oleophila* by Over Expression of Lytic Enzymes				
	Droby, S. Wisniewski M. Goldway, M. Janisiewicz, W.J. Wilson, C.L.	ARO, Min. Ag USDA, ARS MIGAL R&D USDA, ARS USDA, ARS	$310,000	3 years	2000

8 Appendix B: Information providers: Personal communication

Samir Droby – PI of BARD grants, Department of Postharvest Science, ARO, Min, of Agr.

Yoram Cahlon – Marketing Manager for Israel and Palestine Authorities, Syngenta