Institution: Swansea University	
Unit of Assessment: 7 - Earth Systems and Environmental Sciences	
Title of case study: Enabling the widespread use of an insect-killing fungus to control crop pests; from lab to regulator to commercial success.	
1. Summary of the impact	

Research conducted by Professor Butt at Swansea University has led to significant environmental and economic impacts. It has provided evidence critical to the successful registration and commercialisation of the fungus *Metarhizium anisopliae* as a **biocontrol agent in Europe and North America for insect pests**, thereby **enabling a reduced dependency on chemical pesticides**. Insect pests cause £billions of crop losses globally; this is projected to increase due to intensified farming, pesticide resistance and climate change. Many chemical pesticides have been withdrawn due to the risks they pose to human health and the environment, creating a need for benign alternatives. A novel risk assessment showed that the risk to human and animal health was minimal, as metabolites generated by these fungi did not enter the food chain. Furthermore, the data and risk assessment methodology developed have been used by industry and regulatory authorities (such as the European Food Safety Authority) to make informed decisions about the safety of fungal biocontrol agents.

2. Underpinning research

The underpinning research results from a series of EU-funded projects conducted by Prof **Butt** and European partners between 1999 and 2004 to assess the potential health and environmental risks associated with metabolites generated by insect-killing (entomopathogenic) fungi [R1]. In contrast to pesticides, at that time no clear risk assessment guidelines existed for biological control agents (BCAs), impeding their registration and use.

Butt and Research Assistants **Shah** (1999-2006) and **Wang** (1999-2004) characterised the chemical compounds (metabolites) secreted by the insect-killing fungi *Beauveria brongniartii* and *Metarhizium anisopliae*. They established that these fungi, found naturally in soils around the world, produce a vast array of metabolites [R2], and that the range of compounds (e.g. potentially cytotoxic alkaloids and cyclic peptides) produced differed between species and strains. They found that specific metabolites, such as destruxins, can serve as markers of the fungal species. This work was funded by the European Union under [G1]. Regulators responsible for risk assessment and approval of plant protection products require information on all metabolites generated by insect-killing fungi. Given the large number of metabolites produced by the two fungi tested, risk assessment would have been prohibitively expensive, and deterred industry from developing these products. More in depth analysis of the risks of these metabolites was investigated and studies extended to other species of fungal BCAS. This work was funded by the EU [G2]).

Butt and Research Assistants **Skrobek** (2001-2011), Wang and Shah carried out research to determine the potential risk to human and animal health of metabolites generated by *M. anisopliae* and other insect-killing fungi, and to establish whether these metabolites entered the food chain. Specifically, they optimised metabolite isolation methods and evaluated different toxicity testing systems (using protozoa, crustaceans and other invertebrates) to identify the most ecologically sensitive indicator species as an ethical and experimentally tractable alternative to mammalian models [R3, R4]**.** Following this, they investigated the persistence of key fungal metabolites in field trials, residues were undetectable in plant and soil samples even at a 100-fold increased application rate. It was concluded that the metabolites would not enter the food chain and **therefore did not pose a risk to human and animal health** [R3-R5]. It was also shown the insect-killing fungi examined did not produce genotoxic compounds [R6].

The research group then determined the quantity of selected metabolites in the formulated (spore coated rice grain) and unformulated (pure spores) product as well as in the insects treated with *M. anisopliae* and how environmental factors contributed to their degradation in soil samples [R5, R6]. Overall, the research showed that metabolites of *M. anisopliae* and other insect-killing fungi did not enter the food chain or persist in the environment.

3. References to the research

R1 Strasser, H, Abendstein, D, Stuppner, H & **Butt**, T.M. 2000. Monitoring the spatial-temporal distribution of secondary metabolites produced by the entomogenous fungus *Beauveria brongniartii* with particular reference to oosporein. *Mycological Research*. 104: 1227-1233. (Google Scholar - Cited 50 times, IF 2.9)

R2 Strasser, H., Vey, A. & **Butt**, T. M. 2000. Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? *Biocontrol Science and Technology*. 10: 717-735. (Google Scholar - Cited 146 times, IF 0.71)

R3 **Skrobek,** A. & **Butt,** T.M. 2005. Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*. *FEMS Microbiology Letters*. 251: 23–28. (Google Scholar - Cited 24 times, IF 2.05)

R4 **Skrobek,** A. Boss, D. Défago, G. **Butt,** T.M. & Maurhofer, M. 2006. Evaluation of different biological test systems to assess the toxicity of metabolites from fungal biocontrol agents. *Toxicology Letters* 161: 43-52. (Google Scholar - Cited 18 times, IF 3.15)

R5 **Skrobek,** A., **Shah,** A. F. & **Butt**, T.M. 2008. Destruxin production by the entomogenous fungus *Metarhizium anisopliae* in insects and factors influencing their degradation. *BioControl*. 53: 361–373. (Google Scholar - Cited 13 times, IF 2.22)

R6 Kouvelis V.N., **Wang** C., **Skrobek** A., Pappas K.M., Typas, M. A. & **Butt** T.M. 2011. Assessing the cytotoxic and mutagenic effects of secondary metabolites produced by several fungal biological control agents with the Ames assay and the VITOTOX(®) test. *Mutation Research* 722:1-6. (Google Scholar - Cited 3 times, IF 2.22)

Papers R1, R4 and R6 best represent the quality of the underpinning research.

Relevant research grants

G1 *Biological Pest Control (BIPESCO)*; (PI: Butt, EU & Industry Funding; 1999-01; £92,662 of total grant £3,452,000). The EC identified this project as one of the sixteen agricultural success stories under the Fourth Framework Programme (ranked 5th out of 239).

G2 *Risk Assessment of Fungal Biological Control Agents (RAFBCA)*; (PI: Butt, EU & Industry Funding; 2001-04; £284,992 of total grant: £2,627,000). Cited by Government regulatory authorities and industry in registration dossiers/reports, and risk assessment strategy for fungal metabolites adopted by REBECA EU policy support project.

G3 *Development of the entomogenous fungus,* Metarhizium anisopliae, *for the control of vine weevils and western flower thrips in horticultural growing media*; (PI: Butt, DEFRA, Horticulture Development Council & Industry Funding; 2004-06; £346,474).

G4 *Determination of the stability and conidial yield of* M. anisopliae; (PI: Butt, NERC and Koppert; 2004-06; £112,938). Quality control markers validated and used by industry e.g. MycoSolutions Ltd a microenterprise established 2010, produces *M. anisopliae* and other BCAs for industry, academia and US Navy.

G5 *Development of biocontrol agents and strategies for subterranean crop pests (INBIOSOIL)*; (PI: Butt, EU & Industry Funding; 2012-15; £267,000 of total grant: £5,000,000). Prof Butt contributes to the risk assessment of *M. anisopliae* and validation of RAFBCA strategy.

4. Details of the impact

The research has led to the commercialisation of **new pest-control products**. Biological pesticides must be registered and approved by regulatory authorities (e.g., UK Chemicals Regulation Directorate) before they can be sold, and must demonstrate that they adhere to a large number of criteria (including fate in the environment, impact on non-target organisms), and safety requirements before being registered for use on an industrial scale in food production. Prior to the work outlined in Section 2, companies developing plant protection products did not have complete data on the risks posed by insect-killing fungi to support their application for registration and regulators had no information on the risks these new products posed. As a result, food producers and consumers had few alternatives to chemical pesticides. The research findings have **informed the European Commission** on the risk assessment of BCA metabolites generated by insect-killing fungi, enabling the registration of *M. anisopliae* and other insect-killing fungi, leading to their commercialisation and use as an alternative to chemical pesticides.

International reach is evidenced through impact on regulatory authorities: RAFBCA [G2] data demonstrated that by focussing on the crude and selected metabolite extracts the risk assessment process could be simplified, impacting Directive 91/414/EEC and Directive 2001/36/EEC for the registration of biopesticides. The RAFBCA risk assessment strategy for entomopathogenic fungal metabolites, endorsed by REBECA [C2], accelerated the registration process and reduced registration costs by removing the need to test hundreds of metabolites produced by fungal BCAs.

This research led to Draft Assessment Reports in 2007 (on Lecanicillium) and 2008 (on Metarhizium), with **European Food Safety Authority (EFSA) conclusions approved in 2009 and 2011** [C3, C4]. The EFSA conclusions have directly enabled the economic impacts described below.

The Organisation for Economic Cooperation and Development (OECD) was tasked by its 34 member countries to harmonise risk assessment and registration requirements for BCAs. Its recommendations are usually accepted by the 70 non-member partner countries. A 2010 meta-analysis review [C5], which concluded that spores of insect–killing fungi did not persist in the environment, was adopted as a position paper by the OECD BioPesticide Steering Group, which decided to:

> *"refer directly to your work that is contributing to further international harmonization in the assessment of microbial biological control agents"*
> Head of OECD Pesticides Programme

The OECD proposed the review be used as a waiver for the data requirement usually needed for registration to show that fungal biopesticides do not persist in soil [C6], reducing registration time and costs for companies developing fungal BCAs.

> "*We believe your work has helped our members to make considerable progress in the development of entomopathogenic fungi for pest control*"
> Head, International Biological Manufacturers Microbial Professional Group.

***Economic impacts** have been achieved through the research's benefits to businesses and food producers: M. anisopliae* strain Met52 (=F52, BIPESCO 5) was registered by Agrifutur and Novozymes BG in 2008 using RAFBCA [G2] data on the risks of metabolites (including the type, quantity and stability in different production systems). It is currently registered for use to target the black vine weevil (which feed on a wide range of soft fruit and ornamental plants causing damage globally of £several hundred million annually) in 11 European countries, USA and Canada (registered in the UK in 2010) [C10]. Met52 represents 20% of the UK market for the control of this pest in containerised hardy nursery stock, bedding, indoor ornamentals and soft fruit. As a result:

> "*annual European sales increased from **$100K in 2010 to $900K in 2012***"
> Regulatory Specialist, Novozymes BG
>
> "***Over 60,000 m^3 of growing media** in the UK was treated (with Met52) in 2012*"

Technical Manager, Fargro Ltd

During the assessment period, Novozymes BG and Fargro Ltd have invested resources and created jobs, expecting increased sales, more pests targeted for control and registration in more countries (exact figures on jobs and global sales is commercially sensitive information but can be confirmed by C10 and C11)· Total investment has "***to be counted in millions of $US***" [C10]

Research findings arising from [G2] (i.e. metabolites do not pose any risks) were cited in Draft Assessment Reports for the registration of the insect-killing fungus *Verticillium lecanii* (*Lecanicillium muscarium*) [C4], whilst data on metabolites produced by [G1] resulted in the registration of *B. brongniartii*. The research has **provided an alternative to chemical pesticides** that have been withdrawn by the EU, helping growers to comply with new legislation that obliges member states to implement the principles of integrated pest management with preference given to benign alternatives such as insect-killing fungi. In addition to reducing overall pesticide usage and helping food producers to meet supermarket requirements on minimal chemical residues, Met52 is approved for use in organic systems by the Organic Farmers and Growers (reg. UKE0650, 2013).

5. Sources to corroborate the impact (indicative maximum of 10 references)

C1 Marshall, T. 2011. The New Green Revolution, NERC publications (see also: http://tinyurl.com/NERCpublications)

C2 Strasser, H., Typas, M. Altomare, C., **Butt, T.M.** 2007. Annex 7: Position paper on microbial metabolite assessment. In: Strauch, Strasser, Ehlers, Hauschild (eds) 2007. Report on Deliverable 10: Proposals for improved regulatory procedures for microbial BCAs, Regulation of Biological Control Agents (REBECA, EU Specific Support Action SSPE-CT-2005-022709).

C3 EFSA conclusions (approved 2011) on Metarhizium (http://tinyurl.com/metarhizium) arising from the Draft Assessment Report (2008) on the existing active substance *Metarhizium anisopliae var anisopliae* BIPESCO 5/F52 volumes 1-3. Risk assessment provided by the rapporteur Member State The Netherlands for the existing active substance *Metarhizium anisopliae* of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC.

C4 EFSA conclusions (approved 2009) on Lecanicillium (http://tinyurl.com/lecanicillium), arising from the Draft Assessment Report (2007) on the active substance *Lecanicillium muscarium* strain Ve6 volumes 1-3, prepared by the rapporteur Member State the Netherlands in the framework of Directive 91/414/EEC.

C5 Scheepmaker, J.W.A., **Butt, T.M.** 2010. Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. Biocontrol Science and Technology 20: 503-552.

C6 OECD Environment, Health and Safety Publications Series on Pesticides No. 67. OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents. ENV/JM/MONO(2012)1

C7 Senior toxicologist, Ctgb/Board for the Authorisation of Plant Protection Products and Biocides, Dutch Regulatory Authority

C8 Head, OECD Pesticides Programme

C9 Head, Microbial Professional Group, International Biological Manufacturers Association

C10 Regulatory Specialist, Novozymes BG

C11 Technical Manager, Fargro Ltd