Institution: Swansea University		
Unit of Assessment: 7		
Title of case study: Development of an environmentally friendly insect-killing fungus and its volatile metabolites to control globally important crop pests		
Period when the underpinning research was undertaken: 2000–2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Tariq Butt	Professor	1999 - present
James Bull	Associate Professor	2012 - present
Salim Khoja	Technician	2016 - present
Esam Hameed Hummadi	Research Assistant	2015 - 2018
Period when the claimed impact occurred: August 2013 - December 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Many chemical pesticides have been withdrawn or restricted in their use due to the potential harm caused to humans and the environment. Consequently, growers urgently need safe alternatives. Research conducted at Swansea University has resulted in **(A)** the first intellectual property (IP) licencing of a fungal-derived biofumigant (by Certis Europe BV) using volatiles from the insect-pathogenic fungus *Metarhizium brunneum*, **(B)** reregistration and continued sales of this fungus as a biocontrol by Novozymes and Agrifutur, and **(C)** improvement of the European regulatory authority registration process for microbial biopesticides, that now allows companies to more easily register *Metarhizium* and other biological pesticides. Together, the outputs arising from this research have benefitted the environment by providing safer alternatives, and the research has resulted in 2 patents, investment and income generation for biopesticide companies.

2. Underpinning research

Novel fumigant pesticides are much needed since many fumigants have been withdrawn or restricted in use (e.g. methyl bromide, dazomet) because of the harm they cause to humans and the environment. In addition, many pests have developed resistance to fumigants – for example, stored grain pests have become resistant to phosphine.

The underpinning research results from a series of UKRI, EU and Welsh Government-funded projects led by Prof. Butt between 2000–2020. These projects evaluated the market potential of, and environmental risks posed by *Metarhizium brunneum* (formerly *M. anisopliae*) and related species and their secondary metabolites when used in biocontrol, thereby reducing dependency on chemical pesticides.

Methods and tools to identify, quantify and assess the risk for *M. brunneum* secondary metabolites were initially developed with our EU collaborators between 2000-2013 (Risk Assessment of Fungal Biological Control Agents, or RAFBCA [**G1; R1, R2**]). These published studies [**R1, R2**] showed that they did not enter the food chain and therefore did not pose a risk to humans and the environment. A meta-analysis on the environmental fate of insect-pathogenic fungi [**R3**], showed a temporal decline of the fungus and no soil bioaccumulation risk to the environment and non-target vertebrate species. Furthermore, a study of *Metarhizium* secondary metabolites showed that the quantities of destruxins and other relevant secondary metabolites produced *in vivo* were significantly lower than those produced *in vitro* and were, therefore, unlikely to pose health or environmental risks [**R4**].

[**R4**] also provided a critical analysis of the current risk assessment process for microbial secondary metabolites, identifying bottlenecks to registration and presenting solutions.
Butt and colleagues (Khoja, Bull, Hummadi) have demonstrated that *M. brunneum* volatile metabolites have potent pesticidal (e.g., insecticide, nematicide, molluscicide) properties with industrial potential [**R5, R6**]. These volatile metabolites were shown to be short-lived, leave no residues and lack phytotoxicity, offering a safer alternative to conventional chemical fumigants

[**R5, R6**]. Our studies have also shown that these volatiles can be used to sanitize soil (injected into the soil prior to planting) and can repel or eradicate pests such as plant-parasitic nematodes, slugs and snails, wireworm and tortrix moth larvae, all of which cause significant crop losses worldwide. These studies have resulted in IP licenced to Certis Europe BV. We have also shown that formulated and unformulated volatile organic carbons have nematicide, molluscicide and insecticide properties, killing at high doses and repelling at low doses [**R5, R6**].

3. References to the research

All below references have been peer-reviewed and 4 are published in Q1 journals (JCR 2019). Papers have been supported by funding from The European Commission, EPSRC, BBSRC, The Forestry Commission, NERC and Certis Europe BV. [R5] recognised by publisher as top downloaded paper in Pest Management Science journal 2018-2019. Swansea University authors in **bold**.

[R1]. **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. https://10.1016/j.toxlet.2005.07.014

[R2]. 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. https://doi.org/10.1016/j.mrgentox.2011.01.004

[R3]. 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. https://doi.org/10.1080/09583150903545035

[R4]. Scheepmaker, J.W., Busschers, M., Sundh, I., Eilenberg, J. & **Butt, T.M**. (2019) Sense and nonsense of the secondary metabolites data requirements in the EU for beneficial microbial control agents. Biological Control 136:104005. https://doi.org/10.1016/j.biocontrol.2019.104005

[R5]. **Khoja, S., Eltayef, K.M**., Baxter, I., **Bull, J.C., Loveridge, E.J**. & **Butt, T**. (2019) Fungal volatile organic compounds show promise as potent molluscicides. Pest Management Science 75: 3392–3404. https://doi.org/10.1002/ps.5578

[R6]. **Khoja, S., Eltayef, K**., Baxter, I., Myrta, A., **Bull, J.** & **Butt, T**. (2021) Volatiles of the entomopathogenic fungus *Metarhizium brunneum* attract and kill plant parasitic nematodes. Biological Control 152:104472 (N.B. published on-line 4 Nov 2020). https://doi.org/10.1016/j.biocontrol.2020.104472

Relevant Research Grants (T. Butt PI in all cases)

[G1]. Risk Assessment of Fungal Biological Control Agents (RAFBCA); (EU & industry funding; 2001–2004; GBP284,992 of total grant worth: GBP2,627,000).

[G2]. Development of biocontrol agents and strategies for subterranean crop pests (INBIOSOIL); (EU & industry funding; 2012–2015; GBP267,000 of total grant worth GBP5,000,000).

[G3]. Biological pest control (BIPESCO) of insect pests that threaten tree health; (BBSRC & industry funding, within the RCUK–Defra–LWEC tree health and plant biosecurity initiative; 2014–2017; GBP400,363 of total grant worth GBP1,200,000 (GBP900,000 grant from BBSRC plus GBP300,000 from industry)).

[G4]. Development of programmes and strategies for effective wireworm control in European maize (NOVOCONTROL); (Certis Europe Ltd.; 2017–2020; GBP147,700).

[G5]. Nematicidal volatiles from the entomopathogenic fungus *Metarhizium brunneum*; (GBP10,000 from BBSRC plus >GBP25,000 support from Certis Europe BV, 2018–2019).

[G6]. Novel formulations for deployment of biopesticides with fumigatory activity; (Welsh Government ESF and Lisk & Jones Consultants Ltd. 2018–2019; GBP30,000).

Patents (published)

1. Butt, T., Hummadi, E. (inventors) (2019) Use of volatile organic compounds as pesticides. European Patent office, WO2019185826A1, https://bit.ly/3bLbang
2. Butt, T. (inventor) (2020) Use of volatile organic compounds as molluscides. European Patent office, WO2020225149A1, https://bit.ly/2O9s1ry

4. Details of the impact

(A). Licensing of intellectual property to industry for the development of *Metarhizium* volatile metabolites as novel fumigants

Certis Europe BV became interested in our research on *Metarhizium* volatiles in 2016, especially in their ecological role and pesticidal properties. They were particularly interested in the benefits of these volatiles as they were cheaper than current fumigants. Furthermore, *Metarhizium* volatiles improved operator safety and protected the environment due to their ephemeral, non-phytoxic nature. Our studies [**R5, R6**] led to IP that was licensed to Certis Europe BV, who filed two patents (nematicide, molluscicide) of which Prof. Butt was named as the inventor [**C1, C2**]. Influenced by our research, Certis Europe BV strategically prioritized the development (investing > GBP250,000) of the fungal biofumigant aspect of their innovative 'CleanStart' soil pest management programme.

"Certis Europe has recognized the commercial potential of the Metarhizium volatile compounds and has licensed the IP from Swansea University, as well as filing 2 related patents (for nematicides, molluscicides). From Prof Butt's research, which has allowed us to accelerate this line of product development, we know that the volatile compounds will repel pests at low dosages and will stimulate plant growth. This potential increase in plant yield using Metarhizium volatile compounds represents a potential 'game changer' in the way we manage soil pests and we are committed to obtaining regulatory approval on the basis of this research to license the new products (de novo fumigants). This commitment represents a significant financial investment on our part, and a strategic shift in how we future proof our crop protection solutions. We would not have taken such a decision without the strong underpinning evidence that Prof Butt's research has uncovered and we look forward to incorporating Metarhizium volatile compounds into our pest management suite to ensure the best crop yields with minimal human and environmental impact." [Product Development Manager, Certis Europe BV, **C1**].

Based on our findings [**R5, R6**], Certis has developed three new prototype lines based on *Metarhizium* volatiles: 1. A fumigant that kills plant parasitic nematodes (patent published, WO2019185826A1), which cause annual crop losses exceeding USD100,000,000,000 worldwide; 2. A molluscicide (kills slugs and snails) pellet (patent published, WO2020225149A1); and 3. A repellent granule that prevents attack of crops or stored produce by pests such as slugs, snails, and insects.

(B). Registration and sales of new products based on risk assessment of *Metarhizium* Secondary Metabolites

The INBIOSOIL [**G2**] and BIPESCO [**G3**] projects confirmed earlier work (e.g. RAFBCA; **G1**) that neither *M. brunneum* nor its metabolites pose any risk to terrestrial and aquatic non-target invertebrates. These findings published in [**R1-R3**] were used by industry (Novozymes, Agrifutur) in 2016 to reregister (i.e. updating product dossiers, essentially renewal of the product) *M. brunneum* for continued sale, specifically strain F52 and the genetically identical BIPESCO 5, sold as Met52 and GranMet respectively [**C8**]. Agrifutur's CEO noted:

"Since 2014 we have been able to establish an R&D product pipeline around Metarhizium brunneum (namely AGF-Ma5) to control new species of pests such as whitefly, aphids and mites. In 2016 we had to renew our dossier. Our researchers have been able to use the new updated dossier which Prof Butt has contributed to, to consolidate our R&D pipeline, which has preserved

jobs and created revenue. For example, since 2016 our M. brunneum products have grown in sales by 15 % and we have employed 3 new people" [**C3**].
Reregistration allowed distributors of Met52 to continue selling the product and in the case of Fargro, the UK distributor, it has enabled them to focus on new markets for the product, such as the control of the pine weevil, a major forest pest:

"In 2016 when, based in part on Professor Butt's work in generating risk assessment and efficacy data, and showing that M. brunnuem was non-toxic and non-persistent in the environment, Met52 was successfully reregistered for sale in the EU, Fargro was well positioned to continue to market Met52 as an alternative to the broad-spectrum chemical products which had traditionally been used to control vine weevil, a pernicious pest of fruit and ornamental crops". [Fargro Technical Director, **C4**].

(C). Supporting regulatory authorities in reviewing and streamlining the complex registration process for microbial biopesticides

Our research on the secondary metabolites of microbial plant protection agents [**R3**] was cited extensively in the 2018 Organisation for Economic Co-operation and Development (OECD) working document on the risk assessment of secondary metabolites of microbial control agents [**C6**].

The lead risk assessor for The Netherlands, who is also a risk assessor expert to the OECD biopesticides group, together with Prof Butt, reviewed EU guidelines for risk assessment of metabolites of microbial control agents in 2016, identifying historic mistakes and key areas which slowed down the process (e.g. unrealistic data requirements, unclear guidance on data requirements) and made recommendations to expedite the risk assessment of secondary metabolites [**R4**]. Their recommendations were made to industry and regulators. The Dutch expert noted:

"The review was developed with inputs from stakeholders – IBMA and Ctgb (Authorisation of Plant Protection Products and Biocides in The Netherlands). Our suggestions were used by the EU Working Group on Biopesticides in the development of a guidance on metabolites. This guidance was endorsed by the Standing Committee on Plants, Animals, Food and Feed on October 23, 2020 and will apply to applications submitted from 01/11/2021 onwards. Prof Butt has given those who are charged with updating the data requirements for MCAs in the EU tools to evaluate potential production of toxic substances by MCAs, paving the way for quicker registration of microbial pest control agents" [**C5**].

Prof Butt has also worked with the International Biocontrol Manufacturers Association (IBMA) which represents ca. 250 biocontrol technology companies worldwide which aim to develop and sell environmentally friendly biopesticides. The Executive Director of IBMA notes that they have cited his research in:

"*advocating with European regulatory authorities to develop a proportionate risk assessment approach regarding the secondary metabolites (as outlined in several of Prof Butt's research papers) which impacts on the registration of beneficial fungal biocontrol agents….. Prof Butt's data on fungal metabolites, has provided valuable evidence to present a robust and accurate picture of the current gaps and needs in this area of biofungicide registration and has provided a basis for adaptation of the regulation with a view to increasing product availability to farmers"* [**C7**].

Furthermore, they note:

"*We have also used Prof Butt's research as the underpinning principle for Platform 1 of our 2019-2024 collaborative roadmap with the International Federation of Organic Agriculture Movements –EU Regional Group, which outlines our position on bringing biocontrol solutions of natural origin to the market via dedicated proportionate regulation*" [**C7**].

5. Sources to corroborate the impact

[C1]. Letter of support from the Product Development Manager, Certis Europe BV, to verify the Certis investment and patents filed based on our IP.

[C2]. License agreement between Swansea University and Certis Europe BV, 2017. Assignment of patent application and related worldwide rights, 001-0047-00094.

[C3]. Letter from Agrifutur CEO; Agrifutur used RAFBCA findings to register *Metarhizium* as the product GranMet.

[C4]. Letter of support from Fargro Ltd. on sales of *Metarhizium* and our contribution to finding new markets. Fargro is the UK distributor of Met52 for the control of black pine weevil. We showed how this fungus kills pine weevil (adults and juveniles, a major pest in commercial forestry. Met52 offers alternative to cypermethrin which has been withdrawn or restricted in use.

[C5]. Letter from environmental risk assessor, RIVM, National Institute for Public Health and the Environment, the Netherlands. We provided support to RIVM which works closely with OECD and EFSA, resolving critical regulatory issues pertaining to biopesticides such as risk assessment of microbial secondary metabolites - ENV/JM/MONO(2018)33 and ENV/JM/MONO(2018)33/ANN1

[C6]. OECD 2018. Working Document on the Risk Assessment of Secondary Metabolites of Microbial Biocontrol Agents. ENV/JM/MONO(2018)33. https://bit.ly/3dXScwj (Refers to our research findings in ENV/JM/MONO(2018)33 and ENV/JM/MONO(2018)33/ANN1).

[C7]. Letter from IBMA that confirms how we have helped industry by tackling regulatory issues such as risk assessment which have hindered registration and commercialization of fungal biopesticides such as *Metarhizium*.

[C8]. EFSA conclusion document. https://doi.org/10.2903/j.efsa.2020.6274. These are findings of EFSA panel which refers to our work on *Metarhizium brunneum* strains commercialized by Novozymes (F52/Met52) and Agrifutur (BIPESCO 5/GranMet)