Progress report

Our Phase 1 bid had three empirical objectives:

- to **identify strains of entomopathogenic fungus** with the potential to control H. armigera;
- to **clarify whether these isolates can work in Brazilian field conditions**;
- and to **quantify genetic covariances across diverse combinations of fungal isolate and host crop plant**.

In the first few months we have already quantified the biological activity of a number of fungal isolates, demonstrating acceptably high virulence in 5/10 tested strains. The most promising of these strains will soon be exposed to temperature and humidity conditions similar to the Brazilian field; we will have preliminary data in our January progress update. [add figure 1 here]

The quantitative genetic experiment is ongoing; so far we have conducted intensive mass rearing of *Helicoverpa armigera* to establish large populations with substantial genetic variation, and optimized methods for rearing and infecting the experimental subjects. We have shown that six host plants are fully compatible with our rearing and experimental protocols and selected three of these in combination with two fungal isolates (based on their high virulence noted above) to quantify how the relative fitness of the pest changes across different pesticide-crop combinations.

In addition to our empirical objectives, we have begun individual-based modelling to explore how combinations of crop and pathogen heterogeneity can sustain susceptibility (see Challenge 1a in the proposal below), and to illustrate the effects of diversity on resistance management for stakeholders. One important conclusion is that *H. armigera* dispersal is sufficiently extensive that the spatial scale of agricultural heterogeneity needed to impede resistance evolution is large. Crop-biopesticide combinations should be manipulated at field or district scales, rather than requiring individual farmers to subdivide fields: thereby overcoming one substantial practical concern of our proposal's effects on farming operations.

We have also worked closely with partners and stakeholders in planning our second stage bid in order to increase the impact of our work. We conducted pilot telephone surveys that have helped formulate a nationwide online survey of relevant agricultural stakeholders in Brazil on their knowledge and experiences regarding the technological, economic, government and market challenges they face. In September 2018 we met with industrial (from Bayer, FMC corporation, Embrapa) and academic advisers to solicit feedback on our plans. Their advice was instrumental in shaping the current bid. In addition, we held a workshop at the 2018 Brazilian Congress of Entomology and surveyed a wider range of academic, industrial and NGO stakeholders after presenting our work. These data have already shed light on important aspects of farmer and industry priorities, which we have incorporated into our proposal below. [insert figure 2 here]

Research track record of the applicants and specific expertise (and access to any specific infrastructure and equipment required to undertake the stage 2 project) available for the research at the named organisations.

Luc Bussière (Stirling Univ) is an evolutionary biologist with a proven track record in the measurement of selection and evolution in quantitative traits for both laboratory and field systems[1–3]. His previous research has included addressing what maintains genetic variation despite strong selection[4–7]. He has authored several influential papers describing the importance of genotype-by-environment interactions for this process[8–10].

Ricardo Polanczyk (UNESP Jaboticabal) is an entomologist with expertise on pest susceptibility to entomopathogens (mainly fungi and bacteria) and the relationship between these entomopathogens and pest natural enemies (parasitoids and predators)[11–13]. His lab and field research emphasises the role of fungi and bacteria on IPM systems to provide more sustainable agriculture[14–16].

Matthew Tinsley (Stirling Univ) investigates insect host-pathogen interactions from the molecular to the ecological scale. His work demonstrates widespread genetic variation for entomopathogen infection susceptibility in wild insect populations[17] and how immune defence costs maintain this polymorphism[18]. In other work he has uncovered novel impacts of ageing on insect infection resistance[19–21] and studied how inbreeding compromises infection defence[22].

Brad Duthie (Stirling Univ) is a theoretical biologist with a proven track record in modelling complex ecological and evolutionary systems[23,24]. His previous research has included modelling

evolutionary dynamics of inbreeding behaviour given complex genetic architectures and population pedigrees[25–27], ecological interactions among species on spatially explicit landscapes[28], and software development for modelling population management[29].

Belinda Luke (CABI UK) is the Head of the Biopesticides Team at CABI and has twenty two years extensive experience in working with fungi, including on laboratory scale mass production, storage, formulation and quality control aspects, of mainly *Metarhizium* and *Beauveria* species[30–33]. She has worked on two projects that have produced a commercial product, Green Muscle that is registered for use in Africa and a *Beauveria* product that is currently going through EU registration.

Leonardo Fraceto (UNESP Sorocaba) studies the development of micro- and nanocarrier systems for encapsulating compounds with bioactive properties[34–37]. He has previously developed carrier systems for herbicides and other compounds of interest for pest control in agriculture (including for biopesticide fungi)[38], and has patents for technologies that greatly reduce the required concentrations of active compounds. He is currently leading a FAPESP thematic project involving Agriculture, Micro/Nanotechnology and Environment (2017/21004-5).

Renata de Lima (Sorocaba Univ) is an established researcher in Nanotechnology, Mutagenesis and Genetics, with a track-record in measuring the ecotoxicology of nanocomposites, agrochemicals, and biocides[34,35,38,39]. She will provide invaluable expertise on improving the persistence of fungal biopesticides without risking adverse side-effects of the formulations for other organisms.

Rosie Mangan (Stirling Univ) is an applied entomologist working to make pest control more environmentally sensitive and sustainable[40,41]. She has studied factors regulating insect abundance and distribution, population growth, and the ability of insects to function under varying densities, diets, and temperatures[42,43]. She has previously examined the intensive use of chemical pesticides, the prevalence of insecticide resistance genes in major crop pests and the need to develop and deploy strategies that reduce our reliance on chemical pesticides[44].

Yelitza Colmenarez (CABI Brazil) specialises in biological control[45,46], integrated pest management (IPM) and sustainable production[47–50]. She has many years of experience working with multidisciplinary teams, developing international cooperation projects and establishing IPM and biological control programmes for key agricultural pests in Latin America and the Caribbean.

Natália Corniani (CABI Brazil) has expertise in plant biochemistry and physiology. Her work covers mechanisms of action of natural and synthetic compounds and other aspects of agro-chemical research[51–54]. She is an Integrated Crop Management Advisor at CABI Brazil working in international development projects related to biological control and agricultural extension[48,49].

Nils Bunnefeld (Stirling Univ) is an expert in using behavioural games to study conflict resolution and behavioural change, working at the interface of ecology, social science and economics[55–58]. He leads a £1.1m ERC Horizon2020 grant on conflicts between food security and biodiversity conservation. He and his team have invaluable experience and expertise for our project, having already successfully played over 500 games with local farmers in Gabon, Madagascar and Scotland.

Strength of the UK/Brazil scientific collaboration and how it will bring the relevant UK and Brazilian research communities together

Our collaboration brings together experts in several disciplines of basic and applied science to focus on a pernicious worldwide problem (insecticide resistance) that has particular salience for Brazilian agriculture. Our team is uniquely poised to contribute to a solution. It is anchored by a core in the UK and Brazil that has successfully initiated the development of this ambitious new approach during the first six months of our collaboration, encompasses CABI colleagues (partners on the stage 1 bid) as full collaborators in light of their essential role in delivering impact from our work. Furthermore, this core is complemented by new additions to the team that provide necessary expertise in modelling, microtechnology, economics and social science: vital to translate this science into the uptake of an effective and practical agricultural solution.

Details of the proposed research and activities to be conducted within the stage 2 project and how this builds on previous joint work at stage 1, including the proposed programme of work, individual measurable objectives against which you would wish the work to be assessed, the proposed methodology and expected outputs, and the scientific excellence and novelty of the proposal.

Pesticide-resistant herbivores are among the most important and expensive obstacles to food security[59,60]. Brazil's agricultural economy is immensely productive, but the extensive scale of cultivation and widespread deployment of single insect control measures has created serious problems for resistance management[61]. Few originally effective insecticides remain so over time, as target pests almost inevitably evolve resistance. In response to reduced effectiveness, farmers apply higher concentrations of ineffective chemical agents, which can have devastating environmental consequences[62,63]. Brazil urgently needs new technologies that minimise disruptions to natural environments while sustainably safeguarding the ability to protect crops.

Our phase 1 bid brought together insights from fundamental evolutionary science and recent studies of host-pathogen interactions to propose a revolutionary new approach to sustainable pest management. In natural systems, genetic variation can be sustained despite strong selection provided that the modes of action in the selecting agent are diverse, the response is polygenic, and the environmental background in which selection occurs is inconstant[64,65]. In such cases, the map between individual alleles and high fitness is continually shifting. Although these same rules apply to insecticide resistance[66–68], most agricultural systems are insufficiently complex to promote the genotype-by-environment interactions that are central to maintaining diversity[69]. We exploit the fact that biopesticide fungi present complex and diverse challenges to insect pests, along with the fact that some insects feed on multiple crops, to overlay diversity in crop and fungal biopesticide strain. This enhanced diversity in agricultural landscapes has the potential to forestall directional selection for individual resistance alleles in the long term.

In Stage 1 of our project (see progress report above), we initiated a new collaboration and have already demonstrated the potential for diverse fungal strains to kill an important crop pest. In ongoing work, we are studying how the focal strains cope with Brazilian field conditions and quantifying the genetic covariances that should sustain susceptible alleles in diverse landscapes. In the remaining two years of the project, we propose to overcome three challenges related to the establishment of landscape diversity in crops and biopesticides as a real-world insecticide resistance management strategy:

Challenge 1 is to demonstrate that heterogeneous environments produced by crop and fungal biopesticide combinations can delay the evolution of resistance in the long term.

Challenge 2 is to demonstrate that our fungal isolates are suitable for commercial production, that we can deliver them viably in the field, and that they control pests at the field scale.

Challenge 3 is to identify social and economic barriers in the agricultural community to uptake of this pest control system, and to investigate solutions to overcome these barriers.

***Challenge 1*: Solutions for achieving successful long-term sustainable crop protection**

Our Phase 1 grant will deliver a proof-of-concept: by varying biopesticide strains and host crop plants, it is possible to create fluctuating selection landscapes that greatly reduce directional selection on crop pests. However, the genetic architecture of resistance traits can itself evolve; for example, recombination between host genotypes resistant to different fungal strains could lead to cross resistance to multiple biopesticides. Therefore, the enormous evolutionary potential of insect pests justifies research on the long-term sustainability of our proposed system. To address this challenge, we will combine modelling approaches and laboratory studies to measure the long-term stability of the genetic architecture that delays resistance evolution.

a) How landscape scale and diversity sustain susceptibility alleles

To clarify the scale at which diversified biopesticide strain and crop species use can overcome pest resistance in the long-term, we will simulate the full ecological-genetic system using an individual-based model (IBM). This IBM will allow for interactions among discrete individuals (*H. armigera* pests) on a spatially-explicit landscape where key landscape properties (biopesticide application, crop species) vary locally[70]. Individuals have complex genetic architectures that underlie relevant quantitative traits such as biopesticide resistance, feeding efficiency, and dispersal behaviour[71–73]. Landscapes are equally complex, allowing individual traits to interact with the unique set of landscape properties that occur on a landscape cell[28,74,75]. By parameterising pest genetic architecture with real estimates of dispersal[76] and measures of genetic associations from Phase 1a, then simulating different biopesticide application and crop choice regimes on landscape cells, we can use IBMs to model the evolution of biopesticide resistance *in silico*[77]. We have developed a prototype model of the evolution of *H. armigera* biopesticide resistance using a

simplified genetic architecture and landscape. Simulations from this prototype demonstrate that biopesticide and crop heterogeneity can in principle overcome biopesticide resistance (see http://r.rosemckeon.co.uk/helicoverpa/), but these results have yet to be demonstrated with a suitably realistic genetic architecture and landscape properties. This work package will implement the required realism in an expanded model to simulate the long-term evolution of *H. armigera* resistance. In addition to a key paper describing this work, an important output of the model will be an open-source R package, written in R and C, and made accessible to all on GitHub so that external users can modify parameters in line with various agricultural systems and crop pests of interest. The R package will be externally evaluated by the rOpenSci community, including review by two independent experts who will comment on code usability and quality.

b) The stability of genetic architecture over time

In this work package, we will explore whether the application of a single fungal biopesticide leads to rapid pest resistance evolution and assess whether this selective response is slowed or halted under a heterogeneous selection regime. To examine the long-term sustainability of our new biopesticide system, we will conduct laboratory selection experiments, starting with an outbred population collected from no fewer than 100 wild inseminated female *H. armigera*. We will divide their offspring into nine replicate lines, split evenly into three contrasting selection regimes: a control regime, which will be propagated on soya in the absence of fungal biopesticides; a "single biopesticide" regime, in which larvae reared on soya will be exposed to a single *Beauveria* isolate every generation; and a "variable biopesticide and crop" regime, in which each generation of larvae will be split across all possible combinations of soya and maize crop with either *Beauveria* and *Metarhizium* fungus.

After 10 generations of selection (roughly 1 year), we will perform a quantitative genetic experiment to assess the effects of selection regime on changes in the genetic variance-covariance matrix (the **G**-matrix). We will rear a subset of 100 larvae from each line on artificial diet and without biopesticides to relax selection and minimise maternal effects. Within each line, we will then mate ten sires to two dams, and assay the survival ability of up to 40 progeny per dam in each of five environmental combinations: soya with no biopesticide, soya with *Beauveria*, soya with *Metarhizium*, maize with *Beauveria* and maize with *Metarhizium* (12 lines * 20 dams * 200 larvae = 48,000 larvae). The selection experiment will allow us to empirically demonstrate the degree to which the response to selection under a variable selection landscape is weaker than in a consistent landscape. Furthermore, the half-sibling quantitative genetic design will allow us to assess the stability of the **G**-matrix following ten generations of selection by comparing each line's **G**-matrix with the estimate from the stage 1 experiment. We will also measure and compare "conditional evolvabilities" (which quantify the degree to which selection can operate despite stabilising selection on trait combinations) under each of the selection regimes using well established canonical analysis methods[78].

c) The genomic signature of responses to selection

Here we will investigate the genomic basis of resistance variation to fungal biopesticides and determine how sensitive this is to environmental heterogeneity. Our work does not rely on identifying the precise mechanisms of biopesticide resistance; however, we can nevertheless assess whether the genomic architecture of resistance reflects the findings of our quantitative genetic assays. We will identify single nucleotide polymorphisms (SNPs) that are associated with quantitative variation in mortality following biopesticide exposure. We will adopt the novel approach of pool-GWAS, using next generation sequencing on pools of phenotypically similar individuals to conduct a Genome Wide Association Study. We will identify the number, the effect size and the location of genomic regions associated with variation in resistance. We will use *H. armigera* larvae exposed to one of two biopesticides whilst feeding on one of two plant diets (four treatment combinations): this will assess the extent to which the gene regions associated with resistance are consistent between biopesticide-crop plant combinations and to what extent they differ.

Using the outcrossed base population from *Challenge 1b* we will collect larvae from 200 *H. armigera* females. Each female will contribute 16 larvae evenly split across four treatments (*Beauveria* or *Metarhizium* isolate combined with either a soya or maize diet). After fungal exposure, time of larval death will be recorded (or classified as survived after 10 days) and larvae frozen at -80 °C. For each treatment replicate we will create two pools of cadavers separating the most susceptible 20% (fastest dying) and the most resistant 20% (slowest dying/surviving) of the

population. DNA will be extracted from these 32 pools, then libraries prepared for Illumina HiSeq 2000 sequencing generating 150bp paired end reads. We will sequence pools to x50 coverage, undertake standard data quality filtering to identify polymorphic sites, then use the *Popoolation2* analysis platform to find SNPs with significantly differentiated frequencies between the susceptible – resistant pool pairs and compare these between treatment groups.

This experiment will begin to determine the genomic basis of the genetic covariances we have studied elsewhere in this project (Phase 1a). It will inform our modelling work (*Challenge 1a*) and will assist interpretation of the selective responses we observe in *Challenge 1b*. Furthermore, by identifying candidate SNPs which may respond to selection from biopesticide application in agricultural field populations, we will inform future work to develop a DNA-based early warning system for biopesticide resistance evolution that can be used to monitor *H. armigera* in Brazil.

***Challenge 2*: Solutions for achieving successful crop protection technology deployment**

Our phase 1 grant aimed to provide 'proof of principle' that by applying multiple fungal isolate strains to *H. armigera* feeding on multiple crop species, selection for pesticide resistance could be weakened or averted. In this section we will assess the suitability of these isolates for industrial production and field application, examine technological solutions for overcoming constraints on fungal biopesticide effectiveness in Brazilian climatic conditions, and demonstrate field-scale control of Brazilian pests with our newly developed strains.

a) Demonstrating commercial scalability of fungal isolates

Demonstrating that a strain of fungus can kill a target pest is only the first stage in assessing its potential as a pest control method. In addition, we need to demonstrate that the strain can grow in liquid culture, will sporulate, and that spores can be suitably harvested and mixed in formulations that have acceptable storage conditions and shelf life. We will demonstrate that our isolates have the potential to be produced commercially using a two-phase mass production system. We will optimise the liquid broth stage by testing the following: carbon:nitrogen ratios to maximise mycelial growth; growing time of liquid phase; growing temperature, oxygen transfer rate, effect of dilution of liquid seed culture. Next we will concentrate on the solid phase of the mass production process such as: most effective substrate; effects of substrate preparation; moisture content of substrate; growth time; harvesting conidia. Quality control will be carried out on all production runs to ensure that quality is not compromised. We will prepare the most suitable formulation that is compatible with current application methods. Formulating ingredients (such as surfactants) that are readily available in Brazil will be tested to assess effects on conidia viability, formulation stability at a range of temperatures, shelf-life at a range of temperatures, and spray ability of formulations using a Burkhard bench top sprayer. All mass production development will be carried out in a way that is easily transferable to commercial scale production.

One important concern expressed during our stakeholder workshop in Brazil involved potential constraints on the simultaneous use of fungicides (e.g., to control rust or other fungal diseases) and insect control methods, which could disrupt some farm operations. In order to avoid conflicts between fungicide and our biopesticide strains, we will assess whether our strains are susceptible to 10 of the most commonly used fungicides. We will mix the mass-produced fungi or fungal formulations with fungicides at the half, recommended and double dose as a direct tank mix, and also as a contact experiment where leaves (or equivalent) of a test plant are sprayed with fungicide and then applied with the biopesticide product. We will verify the subsequent ability of such mixed formulations to kill *H. armigera* using bioassays as described in *Challenge 2c*.

b) The potential for microencapsulation to increase fungal spore viability

Whilst entomopathogenic fungi have a track record of successful field control of crop pests, their killing power can be reduced by environmental conditions such as high humidity and high UV. We will research novel methods to protect fungal spores from field degradation to extend their field-active lifespan. We will use well-established microparticle technology approaches[42] to evaluate the effect of microencapsulation on the biopersistence of spores in different fields conditions. Because the development of microparticles with fungal spores depends on the optimization of particle preparation, we will use several methods, including physical (spray drying), chemical (polymerization), or physico-chemical (coacervation and ionic gelation) approaches. These diverse methods can also help circumvent the ability of fungi to act as reducing agents, which can interfere with microparticle formation. We will compare the various formulation methods using laboratory

bioassays as well as the field trials described below in *Challenge 2c*.

c) Test formulations for viability under Brazilian field conditions

Having selected strains for commercial potential (*Challenge 2a*) and explored encapsulation as a method for prolonging viability (*Challenge 2b*), we will compare the effectiveness of classic and encapsulated biopesticide formulations against *H. armigera* under field conditions. We will apply these formulations in the field, then collect leaf samples to evaluate biopesticide persistence and subsequent *H. armigera* mortality. We will collect leaf samples at hourly intervals for 3 days after spraying and offer these leaves to first instar *H. armigera* larvae. Subsequently, we will record mortality 3, 7 and 10 days after exposure. We will also evaluate sublethal effects on surviving larvae according to Sedaratian[79]. We will evaluate biopesticide persistence by counting conidia using phase contrast microscopy on leaf samples up to 3 days after treatment. The covariance between mortality and persistence will provide useful information about the role of biopesticide fungi in IPM systems.

Towards the end of the project we will produce our selected formulations on a scale sufficient to undertake efficacy field trials. Our CABI-Brazil and UNESP Jaboticabal farmer network will provide us with contacts of farmers experiencing early-stage *H. armigera* crop damage suitable for biopesticide application. We will recruit a single farmer, incentivising involvement by purchasing their crop regardless of harvest outcome. We will conduct formulation spraying in a randomised block design with a minimum of three treatments: two formulations and a control. We will compare *H. armigera* larval abundance between the three treatments following application to quantify kill-rate efficacy, asking the farmer to continue normal crop management practices up until the point of harvest. During this time we will compare foliage damage, crop growth and ultimate yield between treatment groups. These data will provide unbiased efficacy estimates with which we can approach commercial partners towards the end of the project to secure investment for product development.

***Challenge 3*: Solutions for achieving successful behavioural change in farmer end-users**

Delaying pesticide resistance evolution can be considered a 'tragedy-of-the-commons problem' because the benefits of adopting practices to limit resistance evolution are only realised in the medium to long term, and contingent on cooperation amongst farmers. Sustainable pest control that effectively defeats resistance evolution will require the farming community to adopt novel agricultural practices which are likely to alter operations and/or profitability. The sustainable pest control solution that we propose requires farmers to adopt novel biopesticide products for pest control, spray multiple biopesticide isolates, and, depending on the modelling in *Challenge 1a*, diversify crop species grown on farms. We will estimate direct economic consequences of our proposed approach, and research methods to encourage this behaviour change by investigating short term benefits of our pest control technology that will be experienced by individual farmers; the operational consequences of changes in practice, and the efficacy of regulatory and economic incentives; and the factors that moderate behavioural change by stakeholders in the Brazilian agricultural sector.

a) Non-target effects of fungal biopesticide application

Unlike chemical insecticides, fungal biopesticides have additional biological characteristics that could contribute to the overall performance of integrated pest control. One concern is their potential to negatively impact non-target organisms (such as pest predators) that provide important ecosystem services, although whether these effects are more or less than the non-target effects of chemical pesticides is unclear. In this work package, we will conduct laboratory and field-scale studies to examine the potential for adverse effects of fungal biopesticides on non-target arthropods. Laboratory trials will be conducted to evaluate both direct impacts (mortality and longevity) and indirect consequences (predator feeding, parasitoid efficacy, reproductive rates and reduced prey availability)[80,81] on several non-target beneficial organisms: which are common natural enemies of agricultural pests: *Podisus nigrispinus*, *Trichogramma pretiosum*, and *Coleomegilla maculata*. We will measure responses in each non-target organism after direct exposure to *Beauveria* or *Metarhizium* isolates suitable for commercial production at four doses (10^6,10^7 10^7, 10^9 conidia ml^{-1}) using a spray tower apparatus[82]. Selected natural enemies will also be presented with fungal infected and uninfected *H. armigera* eggs and larvae to determine the feeding behaviour of predators, impact on parasitism by parasitoids, and the subsequent impact of both on developmental time, survival, and fecundity[83–85].

Field studies will examine the effects of fungal biopesticides on the beneficial arthropod community relative to application of conventional chemical insecticides. We will measure the diversity and abundance of beneficial arthropods in crops treated with our fungal biopesticides, conventional insecticides and untreated control crops using field plots arranged in a randomized block design with ten replicates per treatment. The abundance of natural enemies will be surveyed using pan and pitfall traps, sweep-net and vacuum sampling. The abundance, richness, diversity and evenness of natural enemies will be calculated for each treatment, prior to pesticide application and post-application[86]. The *H. armigera* larval population will also be estimated from the numbers of live and dead larvae counts on plants in each plot, and yield from each treatment plot will be recorded. These data can demonstrate the effectiveness of fungal biopesticides in promoting *H. armigera* natural enemy control, reducing *H. armigera* densities, and the extent to which this translates to a reduction in damage levels, and impacts crop yield and quality[87].

b) Predicting operational costs and benefits of diversifying farm operations

In order to quantify the predicted costs (and associated uncertainty) of implementing heterogeneous biopesticide application and crop species cultivation, pilot areas and focus farmer groups will be selected in several Brazilian states with divergent farming practices and traditions (e.g., Goias, Parana, Rio Grande do Sul, Santa Catarina, and São Paulo). The FAO-developed methodology of *Crop Budget* will be applied as a tool for describing, quantifying and determining monetary values of elements of agricultural business[88]. Crop Budgets are a management tool to estimate costs and evaluate cropping alternatives. In this case, with the support of semi-structured interviews, participant observation, market research, and price surveys, we can estimate the costs of alternative agricultural systems, and their effects on farmers' income. On the basis of these estimates, we can quantify the consequences of adopting more sustainable pest control methods for household economy. To further draw inferences about larger regions and communities (rather than only individual farmers), we will need to build databases of crop budgets. Therefore, data collection must follow sampling design and data processing.

Despite the complexity involved in developing multiple databases, it is possible to design profiles of production systems, by exploiting existing knowledge of production system typologies common in a region. Using the methodologies specified above for individual farmers, we will create a profile of types of production system representing each territory. Then, the crop budget will be applied to each profile to generate comparisons of farmers working with monocultures and using conventional pesticides, and farmers working with more diversified crops and using biopesticide applications. In the course of our interviews, we will also gather data in order to evaluate the potential of various incentivising schemes (on the part of biopesticide producers, sellers, or cooperatives) for generating landscape diversity within and among adjacent farms.

c) Factors that promote the uptake of sustainable farming practice

It is crucial that we understand the barriers to uptake our resistance management technologies by the Brazilian farmer community, alongside the potential efficacy of different strategies to overcome these barriers. We will develop experimental games to test incentives presented to farmers and record their farm management decisions. Such games have a proven track record of clarifying how power dynamics, economic incentives, and social networks affect behavioural change in other contexts[55]. We will recruit farmers through CABI Brazil’s extensive network of contacts in farmer cooperatives and our own colleagues in each of the target states, aiming for a sample of 32 participants within each state. The participants will take the role of one of four neighbouring farmers, each of which makes their own decisions regarding land and pest management. Each participant will use a tablet computer connected via a local wifi hub on which they will manage a single 3x3 field landscape consisting of nine fields (for a total of 36 fields across all four farmers). Importantly, the decisions made by one farmer affect not only the yield of their own farm but also influence the yield on neighbouring farms though pest spill, nontarget organism ecosystem services, and effects on resistance evolution.

In the games, farmers can choose for each of their nine fields what crops they grow and what pest management to apply, but the payoffs for decisions are affected by the behaviour of neighbours and simulated dynamics of pests that respond to farmer decisions. The costs can be moderated to examine the effects of various incentivising alternatives (such as subsidies, insurance schemes, or price premiums for sustainable food production) on farmer behaviour, as well as to examine local and demographic influences on behavioural change. The outcomes of this

work will clarify productive avenues for investment in outreach, education, and advocacy in promoting more evolutionarily sustainable pest control.

The potential for improving AMR and insect pest resistance in the Brazilian agriculture and livestock sectors.

Our proposal builds on fundamental and applied science, and promises to produce clear outcomes that address the urgent need for improved insect pest resistance management in a Brazilian context:

- Biopesticides are a valuable emerging tool with tremendous promise for controlling otherwise pernicious pests.We will make them work even better, tailored for Brazil's climate.
- We will introduce cropping regimes and fungal strains to ameliorate resistance management in the long term.
- We will develop our biopesticide products to the point where they are attractive for investment by the agrochemical sector, to facilitate product development.
- By identifying barriers to uptake, providing solutions and working closely with agribusiness and extension officers we will ensure this technology is attractive for adoption in the field.
- Our vision is of agricultural landscapes as mosaics of divergent selection, where the efficiency of individual farmers need not be sacrificed, but the potential for serious pest outbreaks to overcome control measures is nevertheless constrained by diversity within biopesticide-pathogen strains. We believe we can deliver this vision to the Brazilian agricultual sector within the short to medium term after this grant concludes.

References

1 Murray, R. L. *et al. Proc. R. Soc. B Biol. Sci.* 285, 20181525 (2018); **2** Bussière, L. F. *et al. J. Evol. Biol.* 21, 1683–1691 (2008); **3** Brooks, R. *et al. Evolution (N. Y).* 59, 871–880 (2005); **4** Houslay, T. M. *et al. Funct. Ecol.* 31, 728–741 (2016); **5** Thueler, K. *et al. J. Evol. Biol.* 24, 1477–1486 (2011); **6** Marshall, D. J. *et al. Ecology* 89, 2506–2517 (2008); **7** Hunt, J. *et al. Nature* 432, 1024–1027 (2004); **8** Houslay, T. M. *et al.* in *Encyclopedia of Life Sciences* (John Wiley & Sons, Ltd, 2012); **9** Bussière, L. F. *et al. Genetica* 134, 69–78 (2008); **10** Hunt, J. *et al. Trends Ecol. Evol.* 19, 329–333 (2004); **11** Polanczyk, R. A. *et al. Sci. Agric.* 62, 464–468 (2005); **12** Duarte, R. T. *et al. J. Econ. Entomol.* 109, 594–601 (2016); **13** Lobo, K. dos S. *et al. Rev. Bras. Entomol.* 62, 5–12 (2018); **14** José da Silva, R. *et al. Entomol. Gen.* 36, 219–229 (2017); **15** De Bortoli, S. A. *et al. Bacillus thuringiensis and Lysinibacillus sphaericus* 67–77 (2017); **16** SOUZA, C. D. A. S. F. *et al. Rev. Bras. Milho e Sorgo* 16, 52 (2017); **17** Tinsley, M. C. *et al. Parasitology* 132, 767 (2006); **18** Bashir-Tanoli, S. *et al. Funct. Ecol.* 28, 1011–1019 (2014); **19** Kubiak, M. *et al. Sci. Rep.* 7, (2017); **20** Ariani, C. V *et al. Exp. Gerontol.* 61, 47–53 (2015); **21** Mackenzie, D. K. *et al. Exp. Gerontol.* 46, 853–859 (2011); **22** Whitehorn, P. R. *et al. Proc. R. Soc. B Biol. Sci.* 278, 1195–1202 (2010); **23** Duthie, A. *et al. Am. Nat.* 183, 826–841 (2014); **24** Duthie, A. B. *et al. Proc. R. Soc. B Biol. Sci.* 283, 20161845 (2016); **25** Duthie, A. B. *et al. Evolution (N. Y).* 70, 1927–1943 (2016); **26** Duthie, A. B. *et al. Am. Nat.* 188, 651–667 (2016); **27** Duthie, A. B. *et al. J. Evol. Biol.* 31, 31–45 (2017); **28** Duthie, A. B. *et al. Ecol. Modell.* 251, 260–270 (2013); **29** Duthie, A. B. *et al. Methods Ecol. Evol.* (2018); **30** Acheampong, M. A. *et al. Biocontrol Sci. Technol.* 26, 1516–1525 (2016); **31** Luke, B. *et al. Biocontrol Sci. Technol.* 25, 383–398 (2014); **32** Luke, B. M. *et al. Biocontrol Sci. Technol.* 16, 761–766 (2006); **33** Smith, D. (CABI, 2015); **34** Guilger, M. *et al. Sci. Rep.* 7, (2017); **35** Grillo, R. *et al. J. Hazard. Mater.* 278, 163–171 (2014); **36** Grillo, R. *et al. Chemosphere* 119, 608–619 (2015); **37** de Oliveira, J. L. *et al. Biotechnol. Adv.* 32, 1550–1561 (2014); **38** Fraceto, L. F. *et al. J. Chem. Technol. Biotechnol.* 93, 2056–2063 (2018); **39** de Lima, R. *et al. J. Appl. Toxicol.* 32, 867–879 (2012); **40** Mangan, R. *et al. Biol. Control* 64, 143–151 (2013); **41** Earle, W. *et al. Biocontrol Sci. Technol.* 23, 1267–1283 (2013); **42** Mangan, R. *et al. BioControl* 61, 803–813 (2016); **43** Mangan, R. *et al. Entomol. Exp. Appl.* 157, 164–169 (2015); **44** Walsh, L. E. *et al. Irish J. Agric. Food Res.*; **45** Colmenarez, Y. *et al. Acarologia* 54, 433–442 (2014); **46** Luo, S. *et al. Biol. Control* 68, 6–14 (2014); **47** Vasquez, C. *et al. J. Entomol.* 13, 110–121 (2016); **48** Day, R. *et al. Outlooks Pest Manag.* 28, 196–201 (2017); **49** Colmenárez, Y. *et al. Integrated Pest Management (IPM): Environmentally Sound Pest Management* (2016); **50** Vásquez, C. *et al. Exp. Appl. Acarol.* 65, 227–235 (2014); **51** Dayan, F. E. *et al. Weed Sci.* 63, 23–63 (2015); **52** Corniani, N. *et al. PLoS One* 9, e103704 (2014); **53** Cechin, I. *et al. Ciência Rural* 48, (2018); **54** Cerdeira, A. L. *et al. Planta Med.* 80, (2014); **55** Redpath, S. M. *et al. Trends Ecol. Evol.* 33, 415–426 (2018); **56** Baynham-Herd, Z. *et al. Biol. Conserv.* 222, 180–188 (2018); **57** Mason, T. H. E. *et al. Conserv. Lett.* e12460 (2018); **58** Bunnefeld, N. *et al. Proc. Natl. Acad. Sci.* 111, 12964–12965 (2014); **59** Tabashnik, B. E. *et al. J. Econ. Entomol.* 107, 496–507 (2014); **60** Bradshaw, C. J. *et al. Nat. Commun.* 7, 12986 (2016); **61** Oliveira, C. M. *et al. Crop Prot.* 56, 50–54 (2014); **62** Hallmann, C. A. *et al. PLoS One* 12, e0185809 (2017); **63** Nicolopoulou-Stamati, P. *et al. Front. Publ. Heal.* 4, (2016); **64** Hall, A. R. *et al. Ecol Lett* 14, 635–642 (2011); **65** Reznick, D. *et al. Trends Ecol. Evol.* 15, 421–425 (2000); **66** Raymond, B. *et al. Proc R Soc B* 272, 1519–1524 (2005); **67** Jensen, K. *et al. Sci. Rep.* 6, 28731 (2016); **68** Owusu, H. F. *et al. Sci. Rep.* 7, (2017); **69** Cory, J. S. *et al. Evol. Appl.* 5, 455–469 (2012); **70** Bocedi, G. *et al. Methods Ecol. Evol.* 5, 388–396 (2014); **71** Beres, K. A. *Nat. Areas J.* 27, 390–391 (2007); **72** DeAngelis, D. L. *et al. Annu. Rev. Ecol. Evol. Syst.* 36, 147–168 (2005); **73** Duthie, A. C. (Iowa State University); **74** Pe'er, G. *et al. Oikos* no-no (2013); **75** Aben, J. *et al. J. Appl. Ecol.* 51, 693–702 (2014); **76** Jones, C. M. *et al. Mol. Ecol.* 24, 4901–4911 (2015); **77** Slater, R. *et al. Pest Manag Sci* 73, 1364–1372 (2017); **78** Hansen, T. F. *et al. J. Evol. Biol.* 21, 1201–1219 (2008); **79** Sedaratian, A. *et al. J. Appl. Entomol.* 137, 540–549 (2012); **80** Wu, S. *et al. PLoS One* 9, e84732 (2014); **81** Fazeli-Dinan, M. *et al. Int. J. Pest Manag.* 62, 251–260 (2016); **82** Luz, C. *et al. J. Invertebr. Pathol.* 72, 97–103 (1998); **83** Seiedy, M. *et al. Biocontrol Sci. Technol.* 22, 873–882 (2012); **84** Martins, I. C. F. *et al. J. Econ. Entomol.* 107, 933–938 (2014); **85** Magalhães, G. O. *et al.*

Neotrop. Entomol. 44, 521–527 (2015); **86** Marques, L. H. *et al. PLoS One* 13, e0191567 (2018); **87** Bueno, A. F. *et al.* in *SOJA: Manejo Integrado de Insetos e outros Artrópodes-Praga* (eds. Hoffmann-Campo, C. B., Corrêa-Ferreira, B. S. & Moscardi, F.) 493–630 (Embrapa, 2012); **88** FAO. in *Bioenergy and Food Security Rapid Appraisal. User Manual Crop Budget* 42 (2014)