Annual Progress Report – Production Research

Title of Research Project:	Foundation for the management of Bt resistance in *Busseola fusca* populations in South Africa: Surveillance, population genetics and migration patterns
Name and Trading Name of the Institution/Employer:	University of Pretoria
Names of Lead Researchers:	Prof Bernard Slippers Pfano Mbedzi Prof Brett Hurley Prof Johnny van den Berg Mr Roedolf Nieuwenhuis
Contact details of Lead Researcher *E-mail address:* *Phone:*	bernard.slipper@fabi.up.ac.za 0124203938
Duration of Proposed Study:	1 year
Maize Trust funding received for current year:	R210000 + R70 000
Are there changes to the original project proposal (yes/no)	No

1. **Summary of Progress**

This report relates to a larger project, with the overall aim of gaining an understanding of *Busseola fusca* (the maize stalk borer) distribution, population genetic diversity and structure and its migration patterns, in order to consider their implication for spread of Bt resistance genes across populations in different geographic regions and agro-climatic zones. This understanding is the first step for alternative management of *B. fusca* pest in maize, using modern biotechnology techniques such as CRISPR-Cas gene editing.

This part of the project was focused on surveillance of *B. fusca* adults across six provinces in the 2020/2021 season. We originally envisioned the trapping to start at the end of 2020. This was not possible, as funding was not yet available. Supplies of materials and logistics were also complicated by the second wave of COVID-19 early in 2021. Pheromone lures were sourced for *B. fusca*, imported and a pilot study was conducted to confirm efficiency of pheromone lures prior to placement of traps across the provinces between December to February 2020/2021.

Pheromone traps were subsequently set up in March 2021 in Limpopo, North West, Free State, Mpumalanga, Kwa-Zulu Natal and the Eastern Cape to monitor *B. fusca* distribution and capture additional samples for population studies; further to larval and pupal samples collected during field visits and funded separately. At least forty traps were set up per province as was proposed (**Figure 1**). Two maps of *B. fusca* distribution across the six provinces surveyed during April, and May are shown in **Figure 2**. The collected samples were sent to FABI for preservation, DNA extraction and population genetic analysis, which is ongoing.

The pheromone traps were monitored in April and May. Pheromones were replaced after the first sampling in April. More than 4400 moths were captured during this time. A summary of the areas of highest trap catch numbers are shown in **Figure 2**, and more detailed reports of the trap placements and catches are provided as appendixes. Trap catches were lower in May, as expected. A bar graph representation of pest counts from the date of placement until the end of May is shown in **Figure 3**. Catches were not evenly distributed across the country, and shifted during the two survey periods. Mpumalanga had the highest catches, while Limpopo had the lowest during the trapping period. A bar representation of pest numbers per province over the survey period is provided in **Figure 4**.

A related project on 'Population Genetics and Gene Editing of the Africa Maize Stalk Borer, *Busseola fusca',* is separately funded by the Maize Trust and applied for via SANSOR, and reported elsewhere. We will not repeat that report here, but provide a brief summary. The microsatellite marker development has been completed and are being optimized for use on the collected samples. Size polymorphism pre-screening of microsatellites has been done through sequencing samples from Gauteng, and Eastern Cape, as well as a subset of trap samples from Mpumalanga, KwaZulu Natal, and Free State. Each sequenced sample was compared with the Kenyan isolate (from which markers were originally designed) for repeat sequence length polymorphism assessments. Some markers that showed two bands indicating heterozygous samples could not be sequenced through traditional sequencing, but the agarose gels were considered sufficient to demonstrate size polymorphism of these markers. Primers for polymorphic markers were fluorescently labelled *in silico,* and multiplex panels were designed. The labelled primers allow for population screening using fragment length analysis. While the surveillance (which relates to the funding reported here) has been completed, the later than expected start of the project will impact the finalization of the associated population genetics study. Preliminary genetic diversity and population structure were done using COI DNA barcode sequences using a subset of the samples

collected from traps, and this is discussed in details in a separate report also submitted to the maize trust through SANSOR. This part of the project is part of an ongoing PhD study of Pfano Mbedzi that will continue beyond the current funding period.

2. Additional information

Figure 1 Placement of pheromone traps across six provinces of South Africa.

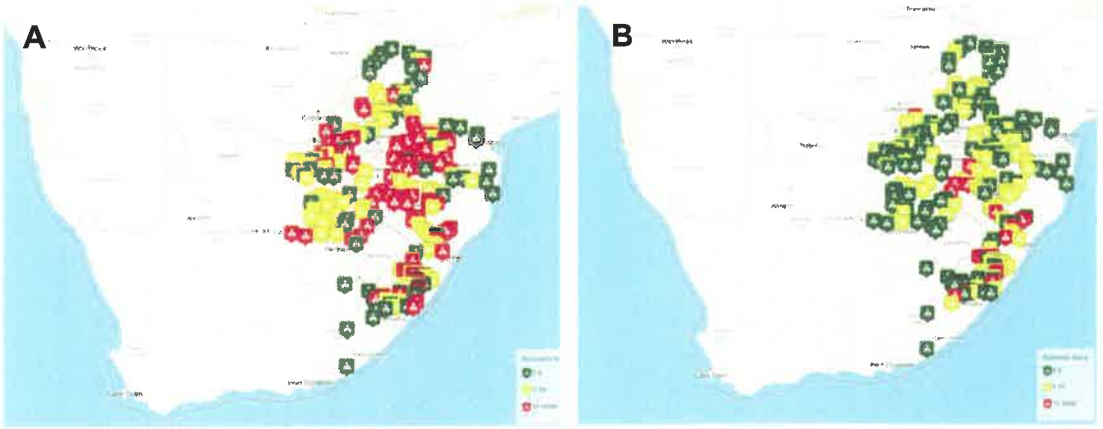

Figure 2 A map representation of *B. fusca* distribution over April (A), and May (B) throughout six provinces.

Figure 3 Heats maps showing varying trap catches between the two sampling periods in April and May. Traps were set up in March, which is why it does not show any catches during that period.

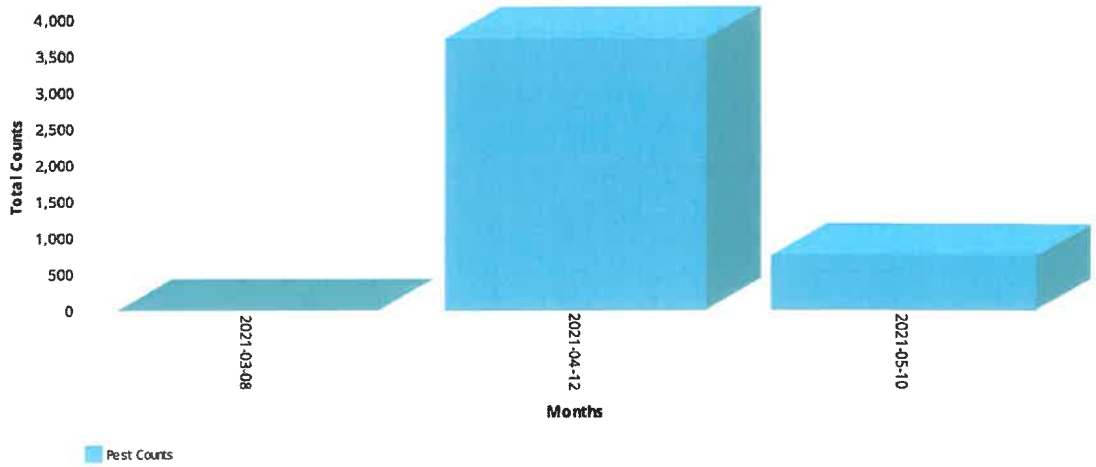

Figure 4 Representation of the number of insects trapped in April and May 2021.

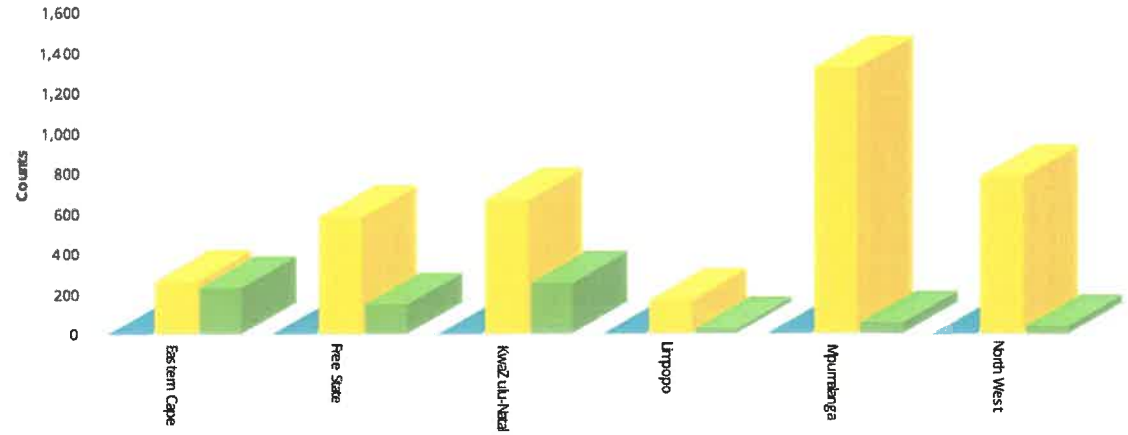

Figure 5 A bar representation of pest counts per province per month. Blue, Yellow, and green bars represent numbers of pests trapped in March, April, and May respectively.

3. Please report on the Deliverables and milestones:

This project deliverables weree to set up and run a surveillance program for *B. fusca* in the 2020/2021 season across six provinces. As reported under points 1 and 2, these objectives were achieved and completed. Pheromone traps were set up in March 2021 in Limpopo, North West, Free State, Mpumalanga, Kwa-Zulu Natal and the Eastern Cape. Traps were monitored in April (with replacement of lures) and May, recording more than 4400 *B. fusca* captures.

4. Changes to project

N/A

5. Scientific Outputs

Scientific papers:	Planned for 2022
Technical reports:	n/a
Articles in industry magazines:	n/a
Conference contributions:	Planned for 2022
Human capacity development:	PhD ongoing: Pfano Mbedzi
Technology transfer:	n/a
Other outputs (Procedures, Methods, Databases, etc):	n/a

6. Personnel / Management / Risk factors that influenced progress and lessons learned (*if applicable*)

N/A

7. Budget and budget justification for the next year of project

Item	Unit costs	Total	Description/justification
Surveillance of traps by Cropwatch	3 x R178250	R534750	3 rounds – placement and subsequent visits for maintenance, recording and sampling of catches and replacement of lures and killing strips (also covers costs of pilot trial)
Materials	240 480 480	R54395	Yellow Bucket Traps Pheromone lures Killing strips
Total expenses		**R589145**	
Costs covered by Maize Trust		R350000*	
Cost covered by leveraged funding		R239145	University of Pretoria and Innovation Africa@UP

8. Conclusions and Comments you wish to share with the Trust

Despite the challenges posed to surveillance programs, general logistics and field work during COVID-19, the proposed surveillance was successfully completed, providing an unprecedented perspective on *B. fusca* distribution and levels across the country. The population genetics and gene editing work, related to Bt resistance screening in *B. fusca* populations continue as part of the PhD project of Pfano Mbedzi.

Importantly, the current surveillance project also provided motivation and methods for developing a more comprehensive national surveillance program for flight patterns and distribution of Maize Lepidoptera. We intend to expand on the current *B. fusca* monitoring project, and link it to screening of *Spodoptera frugiperda* and *Chilo partellus* in parallel. We intend to do this

surveillance for all three insects across the coming season, to get a full season picture of flight patterns of adults and distribution of populations. This information will be connected to both crop and environmental data from a network of farmers and collaborators developed over the past seasons. Further collections will add to the detail of the population genetics studies too. The information will also be invaluable for modelling population dynamics linked to climate variables, for understanding patterns of population spread and Bt resistance structures (linked to the population genetics studies). The information will be available to participating groups in real time, adding information to act on for management.

9. Signature of Project Leader

Bernard Slippers, Pretoria, 30 September 2021

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Name, Signature, Place and Date