Grower Summary

PE031b

Tomato: Phase 3 of an investigation into poor pollination performance by the native bumblebee, *Bombus terrestris audax*

Annual Report 2020

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TOMATO GROWERS' ASSOCIATION

ANNUAL REPORT

To:

AHDB Horticulture

Stoneleigh Park, Kenilworth

Warwickshire, CV8 2LT

Tomato: Phase 3 of an investigation into poor pollination performance by the native bumblebee, *Bombus terrestris audax*

27 January 2021

Rob Jacobson Consultancy — Science into Practice

WARWICK
THE UNIVERSITY OF WARWICK

Project title:	Tomato: Phase 3 of an investigation into poor pollination performance by the native bumblebee, *Bombus terrestris audax*
Project number:	PE 031b
Project leader:	Mr Philip Pearson (TGA Technical Committee Chairman) British Tomato Growers' Association, Pollards Nursery, Lake Lane, Barnham, West Sussex, PO22 0AD
Report:	December 2020
Previous report:	Annual report, April 2020
Key staff:	Dr Rob Jacobson, RJC Ltd Dr David Chandler, Warwick University Mrs Gillian Prince, Warwick University Dr Ken Cockshull, Research Fellow, Warwick University
Location of project:	RJC Ltd, Bramham, West Yorkshire Warwick University, Wellesbourne, Warwickshire
Industry Representative:	Dr Philip Morley (TGA Technical Officer) British Tomato Growers' Association, Pollards Nursery, Lake Lane, Barnham, West Sussex, PO22 0AD
Date project commenced:	1 January 2019
Date project to be completed:	Originally 31 December 2020 (now extended to 31 December 2021)

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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Robert Jacobson

Director

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Signature ..... Date .....

Dr David Chandler,

Associate Professor

School of Life Sciences, Warwick University, Wellesbourne Campus, CV35 9EF

Signature ..... Date .....

Report authorised by:

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Signature ..... Date .....

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TGA Treasurer

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GROWER SUMMARY

Headline

- Studies in 2020 were seriously disrupted by ToBRFV and Covid-19 restrictions.
- Laboratory based work did proceed, albeit at a reduced pace, focussing on developing and refining research techniques which will be applied in the commercial trials planned.
- Work is continuing with a project extension into 2021.

Background

British tomato growers had successfully pollinated their crops with two non-native species of bumblebees (*B. terrestris terrestris* [Btt] and *B. terrestris dalmatinus* [Btd]) for over 27 years when Natural England withdrew permission for their use in unscreened glasshouses. As a consequence, growers had to switch to the British native sub-species, *B. terrestris audax* [Bta]. The aim of this project is to understand why fruit set in commercially-important varieties of UK tomato has been problematic since growers made that switch to Bta.

In 2018, the British Tomato Growers' Association Technical Committee (TGA TC) organised this two year AHDB-funded project to investigate i) relative performance of Bta and Btt/Btd in commercial crops, ii) effect of high temperature on within-hive activity of both Bta and Btt/Btd, and iii) effect of high temperature on tomato pollen production / viability with emphasis on the cultivars most vulnerable to poor fruit set. This project began in January 2019 and, in the first year, focused on Bta colony development, flight activity and flower visitation as well as aspects of tomato flower / pollen development.

Following the first year of this project three elements of the project were identified which required further work to maximise the outcomes of the project to industry, namely:

1. Remote monitoring of bumblebee colonies – Evaluation of the prototype Arnia system highlighted some components which required further refinement prior to being validated in commercial crops.

2. Pollen viability - Several methods of assessing pollen viability reported in historic scientific literature did not prompt germination of pollen from modern tomato cultivars in our 2019 experiments. Therefore additional studies were required to refine and

perfect the techniques prior to continuing with the previously planned work in commercial crops.

3. Variation in Bta colonies - It became apparent during the first year of this project that the trials were confounded by large variations in both the numbers of adult bees in delivered hives, the subsequent development of those colonies and variation in the morphology of adult bees. This variation may be explained by the genetics of the *B. terrestris* used. In order to better understand this source of variation, molecular techniques (as described in Chandler *et al*., 2019) can be used to investigate the genetic structure of samples saved from the key populations in our 2019 studies and should be incorporated into all subsequent studies in commercial crops.

These three elements were discussed during a review of PE 031b attended by the TGA TC, University of Warwick (UW) team and representatives from AHDB in December 2019 where it was agreed they should be considered in future work.

The work programme for 2020 was thwarted first by restrictions imposed to prevent the spread of Tomato Brown Rugose Fruit Virus (ToBRFV) on tomato production sites and then by the national 'lockdown' introduced to reduce the spread of the Covid-19 virus. As a consequence, the project milestones planned for 2020 could not be completed within the original timeframe and the work programme was further modified to focus on the smaller scale laboratory studies identified above. That work began when the first Covid-19 lockdown was eased, albeit at a reduced pace due to restricted access to the University facilities. These results are reported in this report.

Summary

Remote monitoring of bumblebee colonies

‘Arnia Hive Monitors’ originally developed their remote monitoring system (RMS) for honeybees but preliminary studies indicated that the system could also work with much smaller bumblebee colonies. The RMS not only has the potential to provide continual and more detailed information on Bta activity than labour intensive manual counts but could also provide information on hive environment / health; thus providing a valuable tool for future studies. The following three subject areas were identified for further refinement:

Recording of bee hive weights. The load cells used to record weight in individual Arnia units are being recalibrated and the data analysis algorithms adjusted so that all the units perform identically. In addition, units are being calibrated at a range of temperatures and an algorithm produced so that accurate weight readings are recorded independent of ambient temperature.
Temperature probes. Arnia units are being placed in controlled environment chambers at a range of known temperatures so that the consistency and accuracy of temperature recording can be checked.
Recording bumblebee colony sound for behavioural analysis. The Arnia system accurately records sound from within honeybee colonies and uses this to decode different behaviours such as flight and fanning. The algorithms used for this have been adjusted for bumblebee acoustic analysis. However, the small colonies typically observed with Bta in tomato production nurseries have not produced sufficient volume of sound to distinguish within-hive sound from background noise. We have produced data sets comparing living bumblebee colonies with 'dummy' hives (*i.e.* commercial hives without any bees in them). We are also recording the frequency spectrum produced by *B. terrestris* that can be used by Arnia to refine the software used for acoustic analysis.

Pollen viability

Our experiments in project PE 031a indicated that the anthers of an individual tomato flower from cv Piccolo were generally able to produce many more pollen grains than were needed to fertilise all the ovules in the ovary of the same flower. However, it was possible that not all of those pollen grains were viable. If true, this could be contributing to the problem of missed fruit set in cv Piccolo and other modern small fruit varieties. Moreover, tomato is very sensitive to high temperatures, which are known to reduce pollen viability if plants are exposed to elevated temperature conditions. However, the effect of temperature on the viability of pollen of cv Piccolo and other modern small fruit varieties is not known.

Several methods of assessing pollen viability reported in historic scientific literature were tested during 2019 but none prompted germination of pollen from modern tomato cultivars. Three additional methods were tested in 2020. One method was based on solid germination media while the other two methods utilised different types of liquid media (full details of the components of these media are provided in the 'Science Section' of the full project report). The method based on solid media was found to have limitations which compromised accurate interpretation of results. However, the two methods which utilised liquid media were both successful allowing viable and germinating spores to be observed with the assistance of appropriate staining techniques. These methods have the added benefit of minimising any

loss of viability of pollen grains after collection as the samples can be taken directly from the flower to the germination media. The techniques are currently being fine-tuned with work due to be completed before the end of February 2021.

***Genetic structure of populations of* B. terrestris**

While British and mainland European populations of *B. terrestris* do appear to partition into different genetic groups, there is strong evidence of natural genetic mixing between *B. terrestris* in Britain and mainland Europe - probably because the English Channel presents only a minor barrier to bee migration. This means that the distinction between “native” and “non-native” *B. terrestris* is not as simple as that adopted by Natural England. The environmental risks from using commercial *B. terrestris* must take into account the existence of natural genetic mixing and this information should be used to properly underpin the regulation of *B. terrestris* by Natural England.

Other researchers have already reported two distinct genetic groups (known as haplotypes) among *B. terrestris* populations in Britain, Ireland and mainland Europe. Haplotype A was common to Britain and Ireland, while haplotype B was common to mainland Europe as well as some populations in Britain and Ireland. There is evidence that different haplotypes vary in their susceptibility to disease and it is also possible that genetic differences lead to variations in their general vigour. Hence, the apparent difference in the performance of the commercial bumblebees currently classified as Bta and Btt/Btd could also have a genetic basis but not necessarily be linked to their geographical origin.

Work is underway to shed more light on the genetic structure of natural and commercial populations of *B. terrestris*. Our studies have been based on a molecular diagnostic method which has previously shown that the mitochondrial cytochrome oxidase I (COI) gene shows nucleotide polymorphisms that can differentiate between different haplotypes of *B. terrestris.* It is also possible that different genetic primers used for COI amplification and sequencing are able to give finer resolution of haplotype differences. Thus the COI gene sequence is likely to be a useful genetic tool for helping to identify *B. terrestris* sub-species, and to start to look at variation between bees from different sources.

Financial Benefits

Benefits to the British Tomato Industry - TGA members initiated this series of projects to reduce financial losses resulting from production deficit, increased labour and excessive hive input caused by the enforced change to Bta for pollination of UK tomato crops. For example, one tomato grower estimated that poor fruit set cost his business £50k / hectare in 2015. An investment appraisal conducted as part of PE 031 demonstrated a potential payback from the cost of the project to be achieved from just one hectare of crop in one growing season. When extrapolated to the whole industry over a 5 year horizon, the potential cost-benefit of phases 1-3 of PE 031 is greater than 1:250.

Benefits to the wider scientific / horticultural communities – The project is providing data on flower development, pollen production / viability and bumblebee activity which will benefit not only the tomato sector but the principles and findings can be applied to other sectors growing in similar production systems, such as glasshouse grown soft fruit. The further refinement of the Arnia hive monitoring system will provide an invaluable research tool for pollinator studies and will also have the potential to be used by the industry as part of an increasingly digitised growing environment (*i.e.* 'Digital Twinning').

Benefits to UK population – There is now irrefutable evidence of rising temperatures due to climate change. This project will indirectly contribute to our general knowledge by indicating how those changes are likely to impact on native pollinators in outdoor habitats.

Action Points

Due to the disruption caused to the work programme by ToBRFV and Covid-19 in 2020, we are unable to add any further robust action points to those already detailed in previous reports.