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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
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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.

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

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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.

SCIENCE SECTION

Introduction

The aim of this project is to understand why fruit set in commercially-important varieties of UK tomato has been problematic since growers were required to use native bumblebees for pollination.

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 use in unscreened glasshouses in 2015. As a consequence, growers had to switch to the British native sub-species, *B. terrestris audax* [Bta]. The use of Bta in 2015/16 was associated with poor pollination and a high level of mis-set. Several growers suffered such poor results that they reverted to the labour-intensive manual methods of pollination that had not been used since bumblebees were first introduced.

In 2017, the Tomato Growers' Association's Technical Committee (TGA TC) organised an AHDB-funded survey (Project PE 031) to determine the extent of the problem. This survey collected information from 98% of the UK tomato production area and highlighted several issues that were of serious concern to growers (Jacobson, 2017). Most growers believed Bta to be less vigorous than the non-native bumblebees and more likely to fail to provide adequate pollination should any influencing factor (such as high temperature) be sub-optimal. Modern small-fruiting tomato cultivars (*eg* cv Piccolo) were most likely to suffer significant issues with fruit set. In 2018, the TGA TC organised a six month AHDB-funded practical project (PE 031a; Jacobson, 2018) to begin to investigate factors raised by tomato growers in three key subject areas. First, the team discovered that most Bta colonies went into decline soon after placement in tomato crops, which was in stark contrast to previous experience with Btt/Btd colonies. Second, a study of traffic from Bta hives indicated that there was considerably less flight activity in glasshouse tomato crops compared to hives placed outdoors. Finally, a preliminary study to investigate flower development and pollen production in cv Piccolo provided a basis for more detailed experimentation.

Based on the results of all the above studies, the TGA TC organised a two year AHDB-funded project (PE 031b) to further 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 (Jacobson, 2020). Following the first year of the project three elements of the project were identified which required further work to maximise the outcomes of the project to industry, namely:

Remote monitoring of bumblebee colonies – Evaluation of the prototype Arnia system highlighted some components which required further refinement.

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.

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 *Bt* used. In order to get a better understanding of this source of variation, existing molecular techniques (as described by 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.

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.

At the beginning of 2020, the team faced difficulties with the execution of proposed work on commercial nurseries due to restrictions imposed to prevent the spread of Tomato Brown Rugose Fruit Virus (ToBRFV). Plans changed and considerable time was then spent preparing a glasshouse experiment at UW (Wellesbourne Campus) to investigate the effects of high day temperatures on the production and viability of pollen from the variety 'Piccolo'. These preparations included selecting appropriate glasshouse compartments, equipping them to be able to grow tomato plants, organising the supply of young plants of cv Piccolo, as well as writing and agreeing the protocol for the experiment. Unfortunately, this trial was abandoned when the spread of Covid-19 caused the first 'lockdown' to be introduced in March 2020. A decision was made to focus on the smaller scale laboratory studies identified above and reorted herein. That work began when the first lockdown was eased, albeit at a reduced pace due to restricted access to the University facilities.

As a consequence of these disruptions, the project milestones planned for 2020 could not be completed within the original timeframe. A project extension has been granted to extend the project for a further year and incorporates the elements which were identified in the 2019 review meeting listed above.

Materials and Methods

Milestone 1. Refinement and validation of Arnia system

Further refinement and validation of the prototype Arnia remote monitoring system was required before being used in large-scale trials.

Accurate and consistent recording of bee hive weights. Based on initial studies in 2019, different Arnia units have given different readings. The load cells used to record weight in individual Arnia units are being recalibrated and the data analysis algorithms adjusted so that all the Arnia units perform identically. In addition, changes in temperature have affected the readings given by the load cells. Therefore, units are being calibrated at a range of temperatures (10 -30°C) and an algorithm produced so that accurate weight readings are recorded independent of ambient temperature. The weight data from individual Arnia units at different temperatures are being recorded and compiled. Preliminary analysis is being conducted by UW and Arnia Ltd are using the datasets to produce algorithms to recalibrate individual units prior to re-testing by UW.

Calibrate Arnia temperature probes. Arnia units will be placed in controlled environment chambers at different, known temperatures so that the consistency and accuracy of temperature recording can be checked. Arnia Ltd. will use this dataset to recalibrate the Arnia units including alteration to the readouts provided on the Arnia dashboard.

Recording bumblebee colony sound for behavioural analysis. The Arnia system was developed originally for honeybees, where it is able to accurately record sound from within the colony and use this to decode different behaviours such as flight and fanning. The algorithms used for this were adjusted by Arnia for bumblebee acoustic analysis. However, when used on Bta the units recorded background noise. This may have been because the small colonies we have typically observed with Bta in commercial nurseries do not produce sufficient volume of sound to distinguish within-hive sound from background noise. However,

it may also be that the algorithms used by Arnia for acoustic analysis are not able to effectively filter the frequencies produced by bumblebees from background noise and so require modification. Arnia have datasets from this project comparing living bumblebee colonies with 'dummy' hives (*i.e.* commercial hives without any bees in them). We are also providing recordings of the frequency spectrum produced by *B. terrestris* that can be used by Arnia to refine the software used for acoustic analysis. When Arnia have improved the system we will then validate it by comparing Bta colonies with dummy colonies both in controlled environment chambers and in a commercial nursery.

Milestone 2.3.2. Tomato flower and pollen development

Our experiments in 2018 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 (Jacobson, 2018). However, it was possible that not all of the pollen grains were viable: if true, this could be contributing to the problem of missed set in cv Piccolo. Moreover, tomato is very sensitive to high temperatures, which are known to reduce pollen viability if plants are exposed to moderately elevated temperature conditions 7-15 days before anthesis (Sato & Peet, 2005). The effect of temperature on the viability of pollen of cv Piccolo and other modern small fruit varieties is not known. One of the aims of the project is to develop a workable method for quantifying pollen viability to enable the effects of high temperature on cv Piccolo to be quantified. Several methods of assessing pollen viability reported in the historic scientific literature were tried out in 2019. These all involved placing pollen on agar-based media that were reported to enable germination of pollen grains. Hence the proportion of germinated grains in a sample population could be counted over time, which would enable the viability of pollen samples to be quantified. However, we found that none of the media resulted in successful pollen germination. Three additional methods were tested in 2020 and are reported here.

The methods we studied investigated either the use of solid germination media (as described by Karapanos *et al.* (2006)) or liquid media (we used 2 different liquid media, one by Pressman *et al.* (2002) and the second by Paupiere *et al.* (2017)). Pollen was collected from fully reflexed flowers on tomato plants (cv Lizzano), which had been maintained within an environmental growth room at 23 ^{O}C under 16h of natural day light, and its viability assessed within 30 mins. Collected pollen was either smeared over the surface of semi-solid media (50g sucrose, 151g PEG 600, 15g agar, 50mg H_3BO_3, 1L of H_2O) within a 9 cm Petri dish using a paintbrush or added to 0.5ml of liquid media (Medium A = 100g sucrose, 0.124g H_3BO_3, 0.328g $Ca(NO_3)_2.4H_2O$, 0.231g $MgSO_4.7H_2O$, 0.11g KNO_3, 1L of H_2O; Medium B = or

0.99g H_3BO_3, 0.71g $Ca(NO_3)_2.4H_2O$, 0.197g $MgSO_4.7H_2O$, 0.11g KNO_3, 1L of H_2O) within a 2ml Eppendorf tube. Both dishes and tubes were incubated for 6h and 24h at 25^{O}C after which aliquots were stained with either 10µl of Alexander Dye (10ml 95% ethanol, 1ml malachite green (1% solution in 95% ethanol), 54.5 ml distilled H_2O, 25ml glycerol, 5ml Acid fuchsin (1% solution in H_20), 0.5ml Orange G (1% solution in H_20) and 4ml of glacial acetic acid) 1% solution in H_20) or 0.5% acetocarmine stain and examined under the light microscope (magnification x200) for germinated, non-germinated but viable and non-germinated and non-viable pollen grains.

***Milestone 4. Genetic structure of populations of* B. terrestris**

As part of the project, we counted the “traffic” rate of bees flying in and out of Bta hives during the day. The traffic rate for most hives was low, but there were odd hives where the traffic rate was much higher. We also found large variations in both the numbers of adult bees in delivered hives and the subsequent development of those colonies. Furthermore, there was large variation in the morphology (most notably size) of adult bees (Figure 1). This size variation is normal in wild bumblebee colonies, but small bumblebees are not as good foragers as large bees, and hence a high proportion of small bees in a colony could be associated with poor pollination performance.

Figure 1. Size range of adult Bta found in commercial hives.

Some of these issues about bumblebee performance could be related to genetics, and in particular to genetic variation within and between the native and non-native *B. terrestris* sub-species. Our studies in 2020 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.* Moreira *et al*. (2015) reported two distinct 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.

It is also possible that different genetic primers used for COI amplification and sequencing are able to give finer resolution of haplotype differences (Manlik *et al*., 2017). Thus the COI gene sequence is likely to be a useful genetic tool for helping to independently identify *B. terrestris* sub-species.

A sub-sample of bees from commercial hives of Bta and Btt were collected from a tomato nursery and stored at -80 ºC. Individual bees were ground to a fine powder in liquid nitrogen using a pestle and mortar. DNA was extracted from approximately one third of the powder using a DNeasy mini kit (Qiagen). Total DNA samples were quantified using a NanoDrop®ND-1000 spectrophotometer (Thermo Scientific).

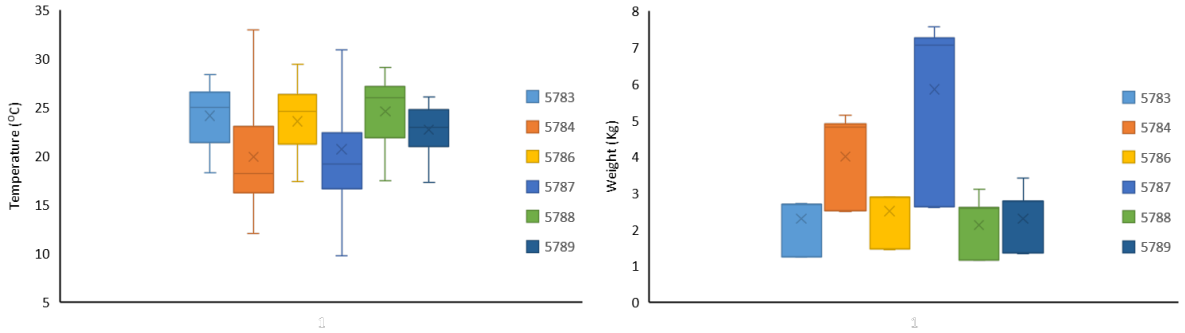
The *B. terrestris* mitochondrial cytochrome oxidase I (COI) gene was amplified by PCR using the universal primers HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3'). PCR amplifications were carried out in a final volume of 20 µl containing 10 µl of REDTaq® ReadyMix™ PCR Reaction Mix (Sigma), 0.5 µM of each forward and reverse primer, and 1 µl of DNA template (1/10 dilution). The PCR conditions were as follows: 94°C for 1 min, 35 cycles of 94°C for 45 s, 52°C for 60 s, and 72°C for 45 s, followed by a final extension of 7 min at 72°C. The PCR products were subjected to electrophoresis on a 1.5 % agarose gel. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) and then sequenced using the forward and reverse primers (Eurofins-GATC). These sequences were compared and consensus versions were constructed. A multiple sequence alignment programme (MegAlign, DNASTAR Inc., Madison, USA) was used to compare the sequences.

Results and Discussion

Milestone 1. Refinement and validation of Arnia system

These studies are underway and are due to be completed before the end of February 2021; *i.e.* prior to trials beginning in commercial crops. An example of the initial data, which displayed the variability in measurements between Arnia units, is shown in Figure 2. This has been sent to Arnia Ltd as part of the recalibration process.

Figure 2. Temperature (left) and weight data (right) from hives with known weight as recorded by six Arnia units within a 20°C controlled environment chamber. Line within the box represents the median and the X represents the mean of the data. The whiskers represent the maximum and minimum.



Milestone 2.3.2. Tomato flower and pollen development

Applying a similar number of pollen grains to the semi solid media proved difficult and they were difficult to see clearly under the microscope. There was no noticeable difference between the two liquid media tested and both were successful; pollen grains could be observed after 6h incubation and staining with Alexander dye showed viable pollen grains (stained purple), viable and germinating pollen grains (stained purple with visible germ tube) and non- viable pollen grains (stained green) (Figure 3). An increased number of pollen grains with longer germ tubes were observed after a further 18h incubation (total of 24h) (Figure 4). This method has the added benefit of minimising any loss of viability of pollen grains after collection as the samples can be collected directly from the flower to the germination media.

Significant progress has been made in the development of a workable method for measuring pollen germination in modern small fruiting tomato cultivars such as cv Piccolo. Further studies are currently underway to optimise the timings of pollen incubation periods and thereby provide a reliable means of quantifying pollen viability.

Figure 3. Viable pollen grains (stained purple) and non-viable pollen grains (stained green) after 6h incubation in liquid media.

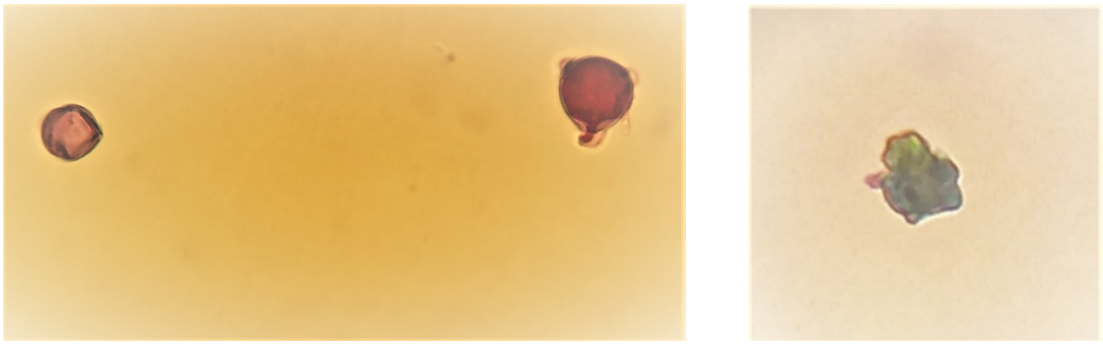
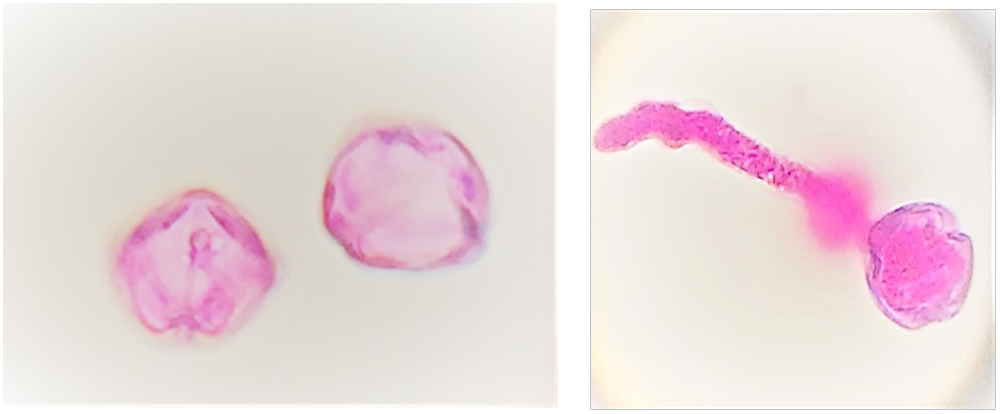


Figure 4. Viable pollen grains with and without germ tube (stained purple) after 24h incubation in liquid media.



***Milestone 4. Genetic structure of populations of* B. terrestris**

The quantity of DNA extracted from an individual bee ranged from 3.5 to 943.2 ng/μl. The majority of the samples produced a PCR product of ca. 850 base pairs (Figure 5). The multiple sequence alignment (Figure 6) showed that there were four regions on the mitochondrial cytochrome oxidase I (COI) gene in which Bta and Btt differed by a single nucleotide.

Work is currently underway to extract DNA from a subsample of bees from all colonies observed in the 2018 and 2019 studies of the project. In parallel to our practical studies, the literature review (Chandler *et al.,* 2019) highlighted variation in the genetic structure of *B. terrestris* populations in the British Isles and on the continental mainland. A number of issues are particularly relevant here:

- *The regulation of "native" and "non-native" B. terrestris for commercial pollination by Natural England.*

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 - this is probably because the English Channel presents only a minor barrier to bee migration (Moreira *et al*., 2015). This means that the distinction between “native” and “non-native” *B. terrestris* is not as simple as that adopted by Natural England.

- *Genetic basis for variation in performance of different B. terrestris sub-species*.
  There is evidence that different genetic groups (known as haplotypes) of *B. terrestris* vary in their susceptibility to disease, including the ability of chronic infections to build up within colonies (Manlik *et al*., 2017). It is probable too that variations between the performance of Bta and Btt/Btd have a genetic basis. Furthermore, we do not know if the populations of Bta used by the different commercial bee producers are genetically similar to each other, or are inbred - both of which could affect performance. It would also be useful to have a method for independently verifying the provenance of native and non-native bees being supplied to UK growers.

Figure 5. Gel image showing 1Kb plus DNA ladder (Invitrogen) and PCR products of individual Bta (lanes 2 – 6), individual Btt (lanes 7-11) and a water control (lane 12).

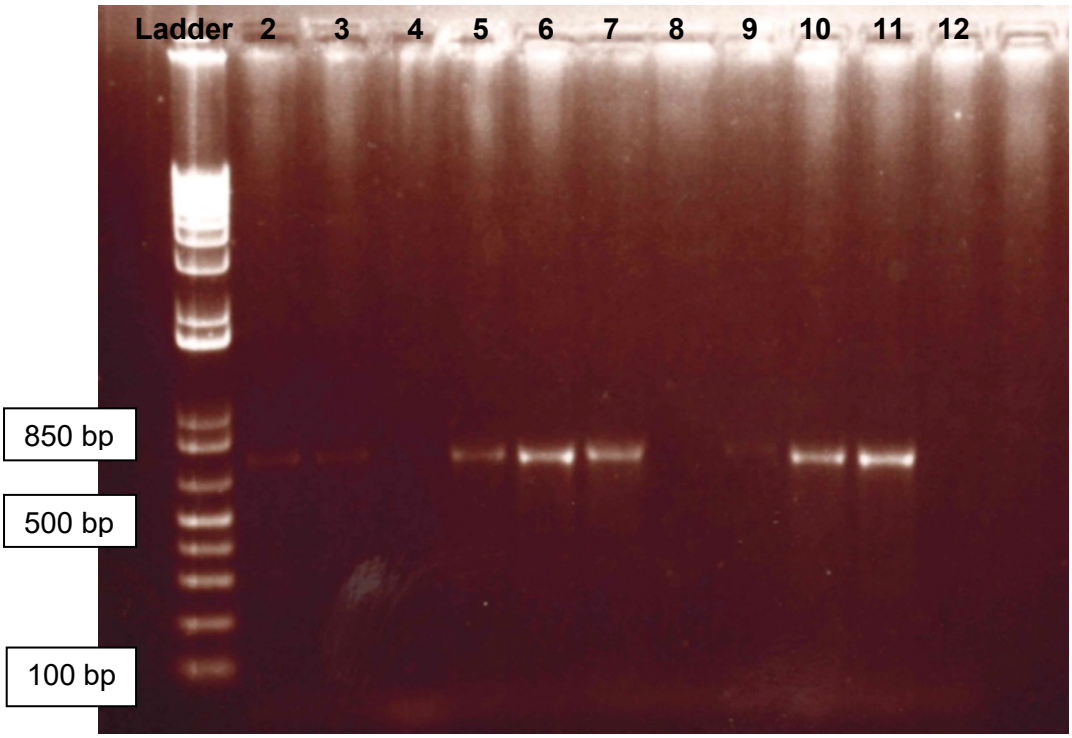
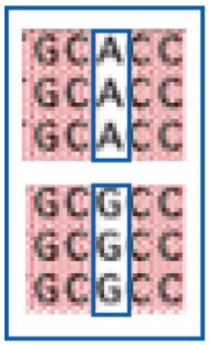


Figure 6. Multiple sequence alignment of the mitochondrial cytochrome oxidase I (COI) gene. The single nucleotide variations are highlighted at locations 122, 293, 467 and 503 in the enclosed boxes.



Location 122

Conclusions

Due to the disruption caused to the work programme by ToBRFV and Covid-19 in 2020, it isn't possible to provide any firm conclusions which add to the robust action points already detailed in previous reports. However, the following points are important as we move into the next phase of the work programme:

- Progress has been made in the recalibration of Arnia units. With further work, this will enable accurate remote monitoring of bumblebee hives within commercial crops.
- Significant progress has been made in the development of a workable method for quantifying pollen viability. With some further refinement, this will enable the effects of high temperature on cv Piccolo to be quantified in commercial crops.
- The distinction between "native" and "non-native" *B. terrestris* is not as simple as that adopted by Natural England and the genetic make-up of these bumblebees needs to be taken into account when evaluating the environmental risks from using commercial *B. terrestris*.

Knowledge and Technology Transfer

- Jacobson (2020). Reports to TGA Technical Committee meetings:

    - Via internet, 3 June 2020
    - Via internet, 2 September 2020
- Jacobson (2020). Project update provided to the British Tomato Working Party via the internet on 16 July 2020
- Jacobson (2020). Pollination issues in 2020. An exchange of topical information with the Tomato Study Group via the internet on 28 July 2020.

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