Grower Summary

PE 031b

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

british

TOMATO GROWERS' ASSOCIATION

FINAL 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*

16 March 2022

Rob Jacobson Consultancy Science into Practice

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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:	Final report, March 2022
Previous report:	Annual report, February 2021
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Location of project:	RJC Ltd, Bramham, West Yorkshire Warwick University, Wellesbourne, Warwickshire R & L Holt Ltd, Springhill Nurseries Ltd, Fladbury, Worcs.
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:	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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GROWER SUMMARY

Headline

- *Bombus terrestris audax* colonies do not perform well in UK tomato crops and this has serious financial implications for growers.
- New evidence will be presented to policy makers with a request to revise the regulations introduced in 2015.
- High air temperatures by day affected production of pollen by tomato flowers in three cultivars. This could become more relevant as air temperatures rise due to climate change.

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 (NE) 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.

British tomato growers are keen to use bumblebees sourced in the British Isles if this can be done without significant economic loss and they have instigated several studies since 2015 in an attempt to improve the situation. Key findings of particular importance to the present project are highlighted here. The full results of those studies can be found in AHDB's series of PE 031b reports and Knowledge Library Pages (Add Link).

The first such study was an AHDB-funded independent review of the scientific literature relevant to the effects of releasing non-native sub-species of bumblebees as pollinators in commercial crops. The resulting peer-reviewed paper (Add Link) concluded that there was insufficient reliable and consistent evidence to support claims that the use of Btt/Btd was harmful to wild populations of *B. terrestris* in the UK. Furthermore, the review reported the genetic structure of wild populations of *B. terrestris* in the UK to be complex with significant differences between populations from different parts of the British Isles. It also highlighted known hybridisation among European 'sub-species'.

In 2017, the Tomato Growers' Association's Technical Committee (TGA TC) organised an in-depth survey of UK tomato growers to gather more precise information about the use of Bta up to that time. Growers representing 98% of the UK production area participated in the survey. In summary, most growers believed Bta to be less vigorous than the non-natives and more likely to fail to provide adequate pollination should any influencing factor be sub-optimal. Modern small-fruiting tomato cultivars (*eg* cv Piccolo) were most likely to suffer significant issues with fruit set, especially during hot weather, but the physiological reason was unknown. The survey was repeated in 2019. It identified a marginal improvement in growers' perception of the performance of Bta, which could have been due to improved breeding stock and/or improved in-crop management of Bta colonies. Nonetheless, growers still considered the performance of Bta to be substantially inferior to Btt/Btd.

There followed a series of short practical studies which made significant progress in subjects related to both the bumblebees and the plants. 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 strongly indicated that there was considerably less flight activity in glasshouse tomato crops than outdoors. However, more detailed work with Bta at that stage was confounded by large variations in the obvious morphology (most notably size) of adult bees and the performance of commercial colonies. The combined evidence from these studies and the literature review began to raise questions about the current classification of *B. terrestris* sub-species and the distinction between 'native' and 'non-native' *B. terrestris*. Molecular genetic studies were then instigated which focussed upon different haplotypes (*i.e.* genetic groups each with its own DNA sequence) rather than sub-species based on geographical origin.

In addition to the bumblebee work, preliminary studies investigated flower development and pollen production in cv Piccolo. This revealed that each flower was usually open on two successive days, although it usually released most of its pollen on the first day with peak pollen release usually occurring between 12:30h and 13:30h. This coincided with the time of peak bumblebee flight activity. The anthers of each cv Piccolo flower had the potential to produce many more pollen grains than were required to fertilise all the ovules in the same flower's ovary. In our experiment, the anther of each flower could produce at least 20,000 pollen grains while the fruit contained fewer than 120 seeds. At that stage, it was not known whether all the grains were viable or whether they would all be released by the actions of bumblebees. Methods of assessing pollen viability reported in the scientific literature did not prompt germination of pollen from modern tomato cultivars so new techniques had to be developed.

Work in 2020 was severely disrupted by restrictions imposed to prevent the spread of Tomato Brown Rugose Fruit Virus (ToBRFV) and the 'lockdown' of research facilities due to Covid-19. All large-scale trials were aborted but the team were able to complete some small-scale laboratory experiments which focused on developing and refining research techniques to be applied in subsequent studies. The present project extension began in January 2021 with studies divided into the following four subject areas.

Summary

Effect of temperature on tomato pollen production and viability

Earlier work had shown that cv Piccolo flowers produced more pollen grains in September than in August which may be due to differences in average temperature. Published data suggested that high temperature had its greatest effect between about 13 days and 7 days before anthesis with the most sensitive period occurring about 9 days before anthesis. The latter being when the pollen mother cells are forming pollen grains. Other factors that might contribute to the setting problems of cv Piccolo could be that this cultivar produces fewer pollen grains than other cultivars when the air temperature rises. In addition, it is possible that not all of the pollen grains that land on the stigma are able to germinate and allow the pollen nucleus to pass along the style to the ovary where the fertilisation of ovules occurs.

Two experiments were done in sequence, each in two fan-ventilated air conditioned glasshouses at Warwick Crop Centre. In each experiment, there were two temperature regimes. The main treatment (HDT) was a high day temperature of 32^{O}C maintained for 12 hours per day followed by a night temperature of 18^{O}C. This was compared to a control with day temperature maintained at 20^{O}C. This was an extreme temperature difference but considered necessary as a 'proof of concept'. If proven, then intermediate temperature regimes could be investigated later. Within each regime, responses in pollen production / viability were recorded in cv Piccolo and compared to cv Duelle (a baby plum tomato) and cv Milandro (a classic round tomato). All flowers were self-pollinated manually, using an 'electric bee', except on the day of, and the day before, sampling for assessment of pollen quantity / quality. The timings of these assessments were carefully planned to provide data from flowers that reached anthesis at critical times in relation to the HDT. Trusses on some plants of each cultivar from each treatment were grown on to produce fruits for seed counts.

The data from the start of the experiments, when flowers had not been exposed to the HDT, showed that all three cultivars produced many more viable pollen grains than were required to fertilise all the ovules in the ovaries of their flowers. No differences were recorded between the HDT and control temperature regimes at that stage. Similarly, there was little or no effect on flowers that reached anthesis on day 21. However, flowers that reached anthesis on day 9 showed a significant reduction in the numbers of seeds per fruit. This effect was amplified in the results from the flowers that reached anthesis on day 15, which showed a dramatic reduction in the number of seeds per fruit leading to the abscission of fruits in all three cultivars. The flowers sampled on day 9 and day 15 had both experienced the HDT between 12 and 7 days before anthesis. This result was consistent with previously published literature which stated that high day temperature could affect fruit set if it occurred 13 to 7 days before anthesis. The effect of the HDT on seed count appeared to be due to an effect on pollen production rather than an effect on pollen viability, or the combined pollen viability index, as pollen viability was relatively high whenever samples were taken.

Genetic structure of populations of B. terrestris.

The decision by NE in 2015 to severely restrict the use of Btt/Btd in the UK was driven by concerns about gynes (sexually reproducing males and females, which are produced at the end of the colony's normal lifespan) 'escaping' from the glasshouse and mating with native British bees to produce a genetic hybrid. However, this rested on the assumption that the *B. terrestris* populations that occur in Great Britain were genetically isolated from the *B. terrestris* bees that occupied mainland Europe.

The project management team felt it was important that we develop in-house molecular genetics methods for *B. terrestris*, with the longer-term aim of providing new data on the population structure of *B. terrestris* that would help decision making and policy development by NE. Studies were initiated to provide DNA sequence data for the *B. terrestris* mitochondrial cytochrome oxidase I (COI) gene, including the identification of nucleotide polymorphisms that can differentiate between different haplotypes of *B. terrestris.*

Our data has shown that there is greater sequence variation in COI than previously reported. While these are preliminary findings, they do suggest that bee 'sub-species' originating from Britain (labelled as Bta) and those from mainland Europe (Btt) do not consist of separate, 'pure' genetic entities, but rather as populations with some haplotypes in common.
This would support the idea that there is already some natural genetic mixing / interactions between populations in Britain and mainland Europe.

Remote monitoring of B. terrestris colonies.

Preliminary studies had indicated that the honeybee remote monitoring system (RMS) produced by 'Arnia Hive Monitors' could be adapted and recalibrated for use with the much smaller commercial bumblebee colonies which would have benefits as an experimental tool and for use in commercial crops. Arnia monitors were set up in a controlled environment room and data sent to Arnia for fine adjustment before the equipment was tested in a commercial tomato crop. However, we were unable to obtain consistent and reliable results from bumblebee colonies within this project.

During 2021, an additional type of bumblebee monitor, produced by Agrolabs, became available and was trialled in a commercial crop. The results with this equipment were more promising and we concluded that this system did have potential to provide high quality, useful data for monitoring bumblebee traffic. However, it would be useful to build the system with temperature and humidity probes so that environmental conditions within hives could be monitored to look for evidence of heat stress effects.

Effect of high temperature on within-hive activity of B. terrestris

Bombus terrestris, collectively thermoregulate their nests in response to cold and heat in order to maintain a relatively constant temperature for brood rearing. In hot conditions, they cool the nest by fanning, which helps maintain a stable temperature for brood rearing. In the wild, the nest temperature is kept within a narrow range independent of the ambient air temperature. For example, brood temperatures measured within a wild *B. terrestris* nest ranged from 31.3 to 33.4 °C despite ambient air temperatures varying from 13.2 to 34.4°C. At extreme ambient conditions, bumblebees spend more time on thermoregulation and less time on other activities such as brood maintenance and foraging. One possible explanation for the poor pollination performance observed with Bta in tomato crops during hot weather is that they are more likely to switch to thermoregulation activities within the hive.

As a first step, a thorough literature search was completed to ensure that we had all the published information on this subject and that information is summarised within the main report. We then ran a set of pilot studies and thereby developed a novel method of observing the effects of elevated temperatures on the behaviour of individual bumblebees. This method was subsequently used to compare the temperature responses of Bta and Btt/Btd.

Our conclusion at this stage is that poor pollination observed with Bta bees is unlikely to be caused by a marked difference in the thermal biology of Bta versus Btt for either (i) the temperature threshold for the fanning response and (ii) the upper lethal temperature for adult bees. We do have some evidence of differences in the proportion of bees that engage in fanning between Bta and Btt, but we would need to perform the same experiment on replicate hives to confirm this.

It is highly unlikely that temperatures within the glasshouse for tomato production will get high enough to kill adult bees. This is not to say, however, that high temperatures are not detrimental to bees within the glasshouse. Temperatures above 32°C are likely to result in bees staying within the hive to fan rather than foraging and could be detrimental to brood.

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. This project has greatly increased the industry's knowledge of the subject and has provided the basis of a case to request NE to reconsider their original decision.

Benefits to the wider scientific / horticultural communities – The project has provided 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 remote hive monitoring systems is providing an invaluable research tool for pollinator studies and could 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

- At the start of this project, it was not clear whether poor fruit set, which resulted when Bta was used to pollinate tomato crops during hot weather, was due to the effect of temperature on plants, bumblebees or an interaction between the two. The findings have greatly improved our understanding and provided evidence to present to policy makers:
- Regarding the plants:
    - The timing of peak pollen release coincides with peak Bta flight activity.
    - A high temperature event reduces pollen production in flowers that are forming pollen grains at that time. This results in poor fruit set from those flowers 7-13 days after the event regardless of the activity of bumblebees. Further studies are required to determine the precise temperature thresholds for different types / cultivars of tomato.
    - These results do not explain the difference in performance between Bta and Btt/Btd.
- Regarding the bumblebees:
    - Bta colonies go into decline soon after placement in tomato crops regardless of environmental conditions.
    - Bta flight activity is less in tomato crops than outdoors.
    - There is no evidence to date to show a marked difference between Bta and Btt/Btd in thermoregulation activities within the hive that would be more detrimental to colony development or to the upper lethal temperature for adult bees.
    - The combined results to date suggest that the tomato plant does not provide a suitable food source for the sustenance of Bta colonies.
- Molecular studies suggest that there is already natural genetic mixing of *B. terrestris* populations in Britain and mainland Europe which questions current classification.
    - Taxonomists should base classification of *B. terrestris* on genetic structure rather than geographical origin.
    - Policy makers should review their decision to restrict the commercial use of *B. terrestris* based on their geographical origin.
    - A meeting has been requested to present the new evidence to NE and Defra.
- The Agrolabs bumblebee remote monitoring system has potential to provide high quality, useful data for monitoring bumblebee traffic but would benefit from the addition of internal temperature and humidity probes to alert growers to adverse conditions.
- High air temperatures by day were shown to affect the production of pollen by tomato flowers in three widely-grown cultivars. Such a response could be a factor adversely affecting fruit set in some cultivars even under present conditions in summer but will evidently become more relevant as ambient air temperatures rise due to climate change.

