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TOMATO GROWERS' ASSOCIATION


# FINAL REPORT

To:

AHDB Horticulture

Stoneleigh Park, Kenilworth

Warwickshire, CV8 2LT

## Tomato: Phase 2 of an investigation into poor pollination performance by the native bumblebee, *Bombus terrestris audax*

30 November 2018

Rob Jacobson Consultancy — Science into Practice

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| **Project title:** | Tomato: Phase 2 of an investigation into poor pollination performance by the native bumblebee, *Bombus terrestris audax* |
| **Project number:** | PE 031a |
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| **Report:** | Final report, 30 November 2018 |
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| **Date project commenced:** | 1 June 2018 |
| **Date project completed:** | 30 November 2017 |

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*[The results and conclusions in this report are based on a series of investigations conducted over a six-month 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

- Most Bta colonies go into decline soon after placement in tomato crops
- Peak pollen flow in cv Piccolo usually occurs around mid-day
- Bta flights in glasshouses are much reduced compared with outdoors and not always well synchronised to peak pollen flow
- A system has been developed to remotely monitor Bta activity within hives

### Background

Bumblebees were introduced to British tomato growers in 1989 via trials in glasshouse crops in West Sussex. The benefits in reduced labour and improved fruit set were so great that by 1992 bumblebees were being used to pollinate all long-season tomato crops in the UK. The system was so reliable that users came to expect perfect fruit set with minimal maintenance.

In the 1980s, the three bumblebee producers tested many populations *of Bombus terrestris* to determine which could be reared most efficiently and which provided the best results in tomato crops. They independently selected two non-native sub-species; *B. terrestris terrestris* (Btt) from central Europe and *B. terrestris dalmatinus* (Btd) from south east Europe. A native sub-species, *B. terrestris audax* (Bta), was tested but dismissed due to inferior performance. In the 27 years since the first release of Btt/Btd in UK tomato crops, there has been no evidence of establishment outside glasshouses or detrimental effect on natural bumblebee populations.

We know that each commercial Btt/Btd colony begins with a single queen producing a small batch of about 8 eggs which she raises to become infertile workers. Thereafter she focuses on producing eggs while the workers take over foraging and brood maintenance tasks. The colony rapidly grows in size and is transferred to the tomato crop about 12 weeks from initiation with 50-60 workers present. Those workers forage among the tomato plants and the colony continues to grow for a further 6-8 weeks. At that point, fertile males and females emerge and leave the hive to mate with adults from other colonies. This marks the end of the cycle.

In 2014, Natural England (NE) produced a document which suggested that non-native bumblebees could escape from glasshouses and hybridise with wild Bta leading to the local extinction of Bta. Following an open consultation, NE revised its policy and permission to use non-native bumblebees in unscreened glasshouses was withdrawn from 31 December 2014. Commercially reared native Bta could still be used without a license. However, the use of Bta in 2015 proved to be far from the reliable and maintenance-free experience to which growers had become accustomed.

British tomato growers are keen to use Bta if this can be done without significant economic loss. In 2017, the British Tomato Growers' Association's Technical Committee (TGA TC) organised an in-depth survey of UK tomato growers to gather more precise information about the situation at that time. Growers representing 98% of the UK production area participated in that survey. Based on those results, the TGA TC organised the present project to investigate:

1. Bta colony life in greenhouses and the impact this has on hive input schedules.
2. Bta biology and behaviour under different environmental conditions with particular emphasis on synchrony between bumblebee foraging and optimum pollen flow.
3. Flower quality and pollen flow in the small fruiting cultivars which now make up 76.9% of UK production.

## Summary


***What was done***

Thirteen growers participated in the survey of Bta colony life in their tomato crops. This provided a spread of locations throughout the main tomato growing areas in the UK as well as different bumblebee suppliers and types of tomatoes. The growers followed an agreed protocol to collectively monitor 161 hives between mid-June and the end of September 2018. During that time, 777 individual colony assessments were completed. An estimate was made of the number of adult bees and the hive was assigned to one of the following indices:

- Index 1 = 1-15 adult bees in view
- Index 2 = 16-30 adult bees in view
- Index 3 = over 31 adult bees in view

The study of Bta biology and behaviour was done at three sites: a small crop of tomatoes (cv Piccolo) at Warwick Crop Centre (WCC), a commercial tomato crop and an outside orchard. 'Bee traffic' was recorded as the rate at which bees entered and exited the hive per hour. This was done by manually counting the numbers of bees entering and exiting the hive every minute from sunrise to sunset. Data were then aggregated as numbers of bees entering / exiting per hour. In addition, an important task was to develop a method of remotely monitoring Bta activity that could be used in future studies. The work was done in

collaboration with 'Arnia Hive Monitors' and involved adapting their honeybee remote monitoring system (HRMS) to work with bumblebee hives. The HRMS records changes in honeybee hive weight, brood temperature, brood humidity, outside temperature, rainfall and bee colony acoustic data. The latter provides an indication of whether the bees are foraging normally or performing other tasks such as 'fanning' to cool the brood. Our challenge was to modify the HRMS to collect similar data from much smaller bumblebee colonies housed in considerably lighter structures of completely different design. Prototypes of the adapted HRMS were tested at the three sites used for manual bee counts. Data was recorded in real time, continuously uploaded to the 'Cloud' via a portal and accessed remotely in the researchers' own offices.

The study of flower quality and pollen flow in small fruiting cultivars was done in a small experimental crop of cv Piccolo at WCC. Plants were grown to commercial standards under the guidance of TGA members. A scale representing discrete and recognisable stages in the development of flowers of cv Piccolo was devised. It comprised Stage 1 (flower buds visible); Stage 2 (petals visible); Stage 3 (bright yellow petals held at right angles to the pedicel); Stage 4 (flower open, petals reflexed); Stage 5 (flower closing, petals becoming pale); Stage 6 (flower closed, petals dying). This scale was then used to monitor the development of flowers along selected trusses initially every hour from dawn until dusk and later every 4 hours from 07:00h to 17:30h each day. The observations were confirmed using continuous time-lapse photography and validated in the commercial tomato crop. Pollen flow was assessed on the same flowers; initially using a simple established method of tapping flowers over a black card and later using a purpose built 'electric bee'. In addition, fruits were removed and their weight, diameter and number of seeds recorded.

***Summary of findings***

Of the monitored Bta colonies, 62% went into decline within 2 weeks of being placed in a tomato crop and this increased to 90% within 4 weeks of delivery (Figure 1). There were variations in numbers of adult bumblebees in hives upon delivery from all suppliers but this was also known to be the case with the non-native bumblebees. The Bta hives generally contained good active colonies upon delivery and we would have expected these to continue to increase in size before completing their cycle. The rapid decline was in contrast to our expectations based on previous studies with the non-native bumblebees. There were no consistent differences between bumblebee supplier, geographical location or tomato type. The only two common factors across all the sites appeared to be Bta bumblebees and tomato crops; thus suggesting that the former either do not like the tomato growing environment or the plants do not provide a satisfactory food source. We must stress that these results relate to the duration of the colonies and do not necessarily mean that the bumblebees failed to provide good fruit set during the monitored period. However, the results do begin to explain why more Bta hives are required than was the case with Btt/Btd and why some growers have obtained improved results by moving to a weekly hive input schedule.

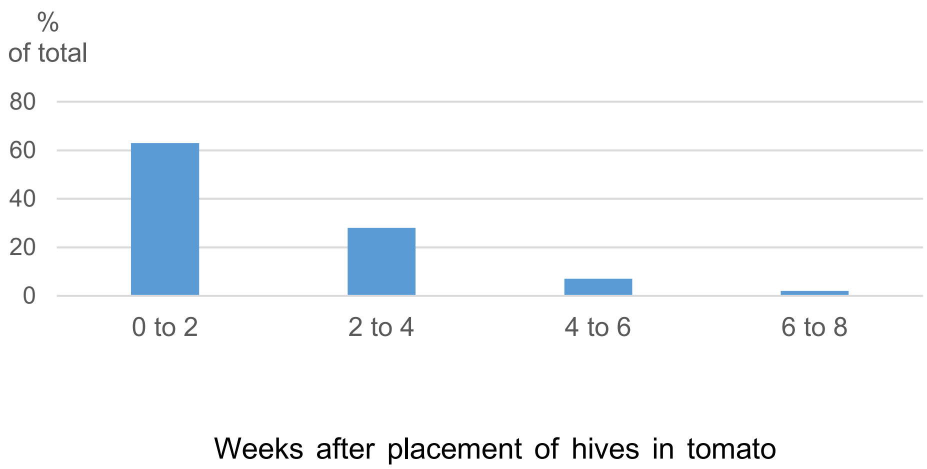



**Figure 1. The time after delivery that 161 Bta colonies, across 12 UK tomato production sites, went into decline after delivery to those sites**

The manual recording of Bta activity showed that adults were generally active over a 12 hour period of the day. Activity followed a similar pattern regardless of hive location, temperature or time of year, with the first activity seen just after sunrise, rising to a peak in activity between 11:00h and 14:00h followed by a fall in activity around 18:00h. Two short peaks of daily activity were often recorded in colonies within the tomato crops but only one, albeit more prolonged, peak was observed in hives kept outdoors. One very notable feature was that activity in colonies kept outdoors was considerably higher than that observed when kept in the glasshouse tomato crops (Figure 2).

The Arnia remote hive monitors not only provided continual and more detailed information on hive activity than the time consuming, labour intensive manual counts but they also provided in depth information on the hive environment / health and will provide a valuable tool for future studies. The collected data showed that brood and glasshouse temperatures never exceeded 30°C and there was no evidence that the recorded flight activity was correlated with temperature. Available literature suggests that *B. terrestris* will generally only cease to forage at temperatures above 32°C.

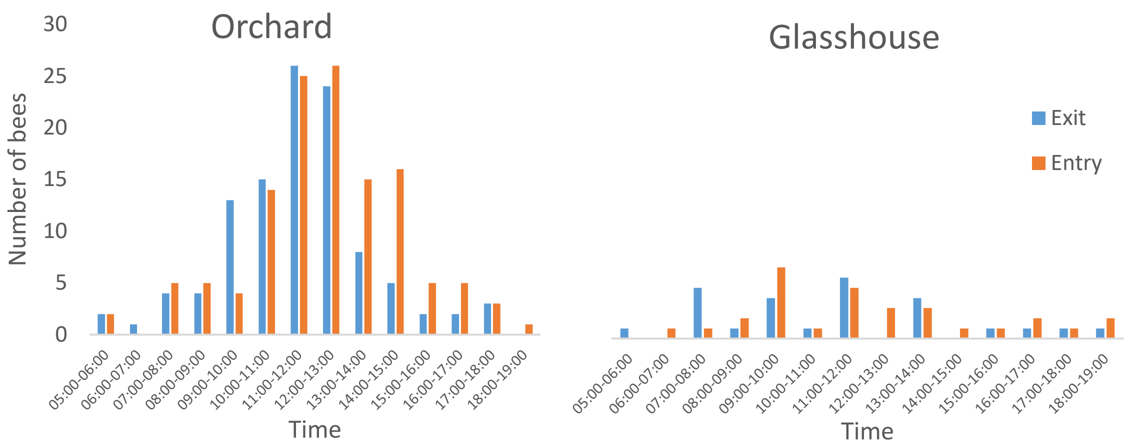



**Figure 2. Comparison of Bta activity in a tomato crop and outside – expressed as numbers of bees entering and exiting the hives 14 August 2018**

Our study of flower development and pollen flow in cv Piccolo revealed that each flower was usually open on two successive days although it usually released most of its pollen on the first day. The peak of pollen release usually occurred between 12:30h and 13:30h each afternoon but, for some flowers the peak occurred much earlier and for others it occurred much later. The tomato flower is normally self-pollinated and it was evident that 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. Although it appeared that each flower produced and released a vast excess of pollen when the flower was shaken with the mini electric bee, further work is required to establish that similar amounts would be released by the actions of bumblebees or by the actions of growers.

Our observations suggest that pollen production and pollen release are inhibited by high day temperatures of 29$^0$C or more which is also the conclusion reached in the published literature. High day temperatures may also have contributed to the variability in pollen production by different flowers, as that was more noticeable in August than in September, and the high temperature may also have affected pollen viability. It is therefore important to establish the proportion of the pollen grains that are viable and able to germinate.

Although various factors could contribute to the failure to transfer enough viable pollen to the stigmas of tomato flowers, a major cause could be that the bumblebees fail to visit all of the flowers at a time when they are each producing adequate quantities of viable pollen and may even be a failure of the bumblebees to release the pollen from the flower's anthers.

## Financial Benefits

The economics of tomato production in the UK have changed considerably since bumblebees were first introduced for pollination. Pressure from retail customers has greatly reduced financial margins and growers have become dependent upon the benefits that are obtained from using biological pollination. It is difficult to generalise about the financial value of British tomato crops due to the wide range of products supplied to retail customers. However, if we assume the farm gate value to be about £850k / ha / season, then the total value of the British crop is about £162m / season. Long season tomato plants produce 35-40 trusses per season. The loss of set due to inadequate pollination on just two trusses equates to about 5.3% of annual production which is in the region of £45k / ha / season. The equivalent losses across the British industry would be over £8.6m.

## Action Points

Projects PE031 and PE031a have advanced the TGA's (*i.e.* the UK tomato industry's) understanding of the poor pollination performance of Bta in tomato crops with particular emphasis on the small fruited cultivars, such as cv Piccolo, which made up 76.9% of UK production in 2016. We have now developed the methods required to obtain more detailed information on how Bta performs within a commercial tomato crop. This knowledge provides a solid foundation upon which the TGA can progress towards our ultimate objective; *i.e.* to reduce financial losses resulting from production deficit, increased labour and excessive input caused by the enforced change from non-native bumblebees to Bta for pollination of UK tomato crops.

In the short term, growers can:

- Liaise with their bumblebee supplier to produce a hive input programme that compensates for the shorter colony life of Bta bumblebees.
- Monitor Bta foraging activity around mid-day, when most open flowers have peak pollen flow, and supplement with manual pollination when there is little activity.

# SCIENCE SECTION

## Introduction

Bumblebees were first introduced to British tomato growers in 1989 via trials in glasshouse crops in West Sussex (Hayman & Walker, 1989). The benefits, in terms of reduced labour and improved fruit set, were so great that by 1992 bumblebees were being used to pollinate virtually all long-season tomato crops in the UK. There followed a few revisions to hive design and some tweaks to hive placement programmes, but the pollination system was so reliable that growers came to expect perfect fruit set with minimal maintenance.

In the 1980s, the three commercial bumblebee producers tested many populations *of Bombus terrestris* to determine which could be reared most efficiently in culture and which provided the best results in tomato crops. They independently selected two non-native sub-species; *B. terrestris terrestris* (Btt) from central Europe and *B. terrestris dalmatinus* (Btd) from south east Europe. At least one of those producers, Brinkman Bunting Bumblebees BV, tested and dismissed the British native sub-species, *B. terrestris audax* (Bta) at that stage due to inferior performance (Griffiths, pers. com. 1990). In the 27 years since the first release of Btt/Btd in UK tomato crops, there has been no evidence of their establishment outside glasshouses or any detrimental effect on natural bumblebee populations.

There is evidence of hybridisation of Btt and Btd where overlapping ranges exist in mainland Europe (Estoup *et al.*, 1996). Given the difficulty in distinguishing sub-species using morphological features, it is possible that further mixtures leading to hybridisation have occurred in commercial cultures. For these reasons, there will be no attempt to distinguish between Btt and Btd in this report.

In the early 1990s, when the commercial use of bumblebees in tomato crops was still in its infancy, the three production companies devoted considerable resource to monitoring Btt/Btd colony development in their clients' glasshouses. Their results were commercially sensitive and were not published. However, one of the authors is able to provide an insight into the findings of one of the producers. Figure 1 shows a typical 'trend' in the life of a Btt/Btd colony, from its initiation in the bumblebee production unit to its demise in the commercial crop. This is based on information from Bunting Brinkman Bumblebees BV (Griffiths, pers. com. 1992; Jacobson, unpublished data 1990-1993). The colonies began with a single queen producing a small batch of about 8 eggs which she raised to become infertile workers. Thereafter she focused on producing eggs while the workers took over all foraging and brood maintenance tasks. The colony rapidly grew in size and would be transferred to the tomato crop about 12 weeks from initiation with 50-60 workers present. The workers then foraged among the tomato plants and the colony continued to grow for a further 6-8 weeks. At around that point, fertile males and females emerged which left the hive to mate with adults from other colonies. This marked the end of the cycle and the colony rapidly declined.

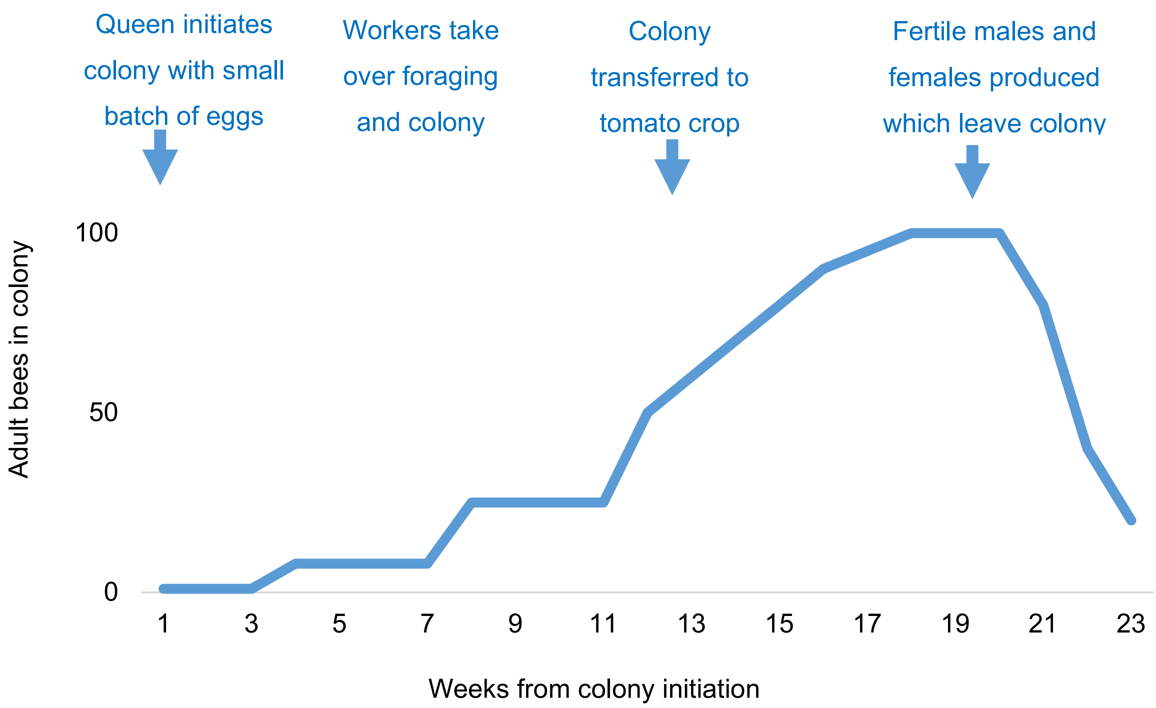



**Figure 1. Typical Btt/Btd colony development from initiation in the bumblebee production unit to its demise in a tomato crop (NB: This chart illustrates the overall trend but the actual numbers of adult bees present were inherently variable and should not be quoted from this diagram).**

In 2014, Natural England (NE) produced a document which suggested that non-native bumblebees could escape from glasshouses and hybridise with wild Bta leading to the local extinction of Bta. In addition, NE proposed that the use of non-native sub-species could lead to the transfer of harmful parasites / pathogens from commercially reared *Bombus terrestris* to wild bumblebees in the UK. Following an open consultation, NE revised its policy and permission to use non-native bumblebees in unscreened glasshouses was withdrawn from 31 December 2014. Commercially reared native Bta could still be used without a license.

The use of Bta in 2015 proved to be far from the reliable and maintenance-free experience to which growers had become accustomed. In fact, 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.

British tomato growers are keen to use Bta if this can be done without significant economic loss. In 2017, the TGA Technical Committee organised an appropriate team to conduct an in-depth survey of UK tomato growers to gather more precise information about the situation at that time. Growers representing 98% of the UK production area participated in that survey. The full results may be found in the AHDB report (Jacobson, 2017) and summarised in a subsequent article (Jacobson, 2018). Key points raised by the interviewed growers were:

- 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.
- 47% of growers thought that Bta colony life was shorter in the tomato crop than they had previously observed with non-natives. However, no one had quantified this.
- There were usually at least 20% more Bta hives than Btt/Btd hives used per hectare during the growing season. 28% of growers said they occasionally ordered extra Bta hives while 69% said this was a frequent requirement.
- Many growers reported that small fruiting tomato 'types' were the most commonly affected by poor set despite the use of extra Bta hives. This prompted an additional investigation into the overall UK change to such cultivars during the period that NE had enforced the switch from Btt/Btd to Bta. In 2011, only 28.8% of UK tomato production was of the cherry / cocktail type (including both loose and vine harvested produce) but by 2016 (*i.e.* post Bta) that had increased to 76.9%.
- About one third of growers said that their staff had asked "*Where are the bees*?" at some point during the season. This related to the apparent lack of Bta activity during normal working hours. One grower believed that this was because Bta forage very early in the morning so that their activity could easily be underestimated. If correct, then Bta activity may not be very well synchronised to peak pollen release / flow in tomato flowers.

Based on the results of this survey, the TGA TC organised the present project to investigate:

- Bta colony life in greenhouses and the impact this has on hive input schedules and frequency of hive deliveries.
- Bta biology and behaviour under different environmental conditions with particular emphasis on synchrony between bumblebee foraging and optimum pollen flow.

- The influence of environmental conditions on flower quality and pollen flow in the small fruiting cultivars which made up 76.9% of UK production in 2016.

## Materials and methods

### Objective 1. Survey of bumblebee colony duration

Details of participating tomato growers are shown in Table 1. It must be stressed that not all growers wanted to be identified in the AHDB report, so the participants are referred to by a site number rather than by name. The participants were all volunteers who provided a spread of locations throughout the main tomato growing areas in the UK as well as different bumblebee suppliers (coded A, B or C) and types of tomatoes. Due to the nature of the recruitment process, the survey organisers were unable to select equal numbers of sites under each of these categories. Nonetheless, most of the important factors were included to some extent. Participating growers monitored variable numbers of hives depending on the resource they were able to devote to the task. There were originally 13 sites but this was reduced to 12 during the course of the study. They collectively monitored 161 hives between weeks 25 2018 and 39 2018. During that time, 777 individual colony assessments were completed.

**Table 1. Tomato growers participating in the bumblebee colony duration survey**

| Site reference (Supplier) | Location | Tomato type / cultivar | Number of: | | Week number 2018: | |
|---|---|---|---|---|---|---|
| | | | hives | assessments | Work started | Work finished |
| 1 (C) | North east | Classic round | 40 | 201 | 26 | 38 |
| 2 (A) | Yorkshire | Classic round | 16 | 54 | 26 | 34 |
| 3 (C) | North West | Piccolo | 4 | 24 | 27 | 37 |
| 4 (C) | North West | Mini plum | 3 | 13 | 29 | 37 |
| 5 (C) | North West | Piccolo | 4 | 20 | 27 | 35 |
| 6 (C) | North West | Cherry, Mini plum, Tomkin | 10 | 55 | 27 | 37 |
| 7 (B) | North West | Piccolo | 4 | 20 | 28 | 36 |
| 9 (B) | Lea Valley | Baby plum, Piccolo, San Marzano | 18 | 72 | 33 | 39 |
| 10 (A) | South Coast | Cherry & Classic round | 16 | 72 | 27 | 35 |
| 11 (B) | South Coast | Baby plum | 10 | 40 | 27 | 33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 (B) | South Coast | Cherry | 10 | 70 | 25 | 37 |
| 13 (B) | Kent | Piccolo | 26 | 136 | 25 | 37 |
| Total | National | | 161 | 777 | 25-33 | 33-39 |

There were two levels of assessment. In the first, one of the project team personally monitored hives at two sites (*i.e.* sites 1 and 2). For each hive, assessments began at the week of delivery and continued at 1-2 week intervals between weeks 26 and 38. At each assessment, an estimate was made of the activity of adult bees within the hive. This was based on the number of adults counted above the insulating wadding (see method below). In addition, each hive was weighed to provide an indication of how much sugar solution had been used from the internal supply.

The second type of assessment involved all the participating growers. In this case, the assessments were simplified to limit any inherent variation between the individual assessors. A protocol was developed in a preliminary study between weeks 17 and 21 2018. In that preliminary study, 22 hives were examined repeatedly giving a total of 57 assessments. The colony assessments were completed between the week of delivery to the nursery and 17 weeks post-delivery; thus ensuring the inclusion of a wide range of activity levels. A draft protocol based on these observations was prepared and circulated to participating growers for evaluation and comment. The final version of the protocol was circulated in week 26 and the growers began to monitor their hives thereafter.

The technique involved switching the hive entrance to the 'one-way' position during the evening so that all bees were collected and held within the hive overnight. The hives were then examined as early as possible the following morning. The cardboard lid of the hive was opened to reveal the brood chamber and then the sides of the hive were tapped vigorously 3-4 times to bring the bees to the surface of the wadding. An estimate was made of the number of adult bees in view and this was assigned to one of the following indices:

- Index 0 = No adult bees in view
- Index 1 = 1-15 adult bees in view
- Index 2 = 16-30 adult bees in view
- Index 3 = over 31 adult bees in view

Despite the disturbance to the hive, it was recognised that a proportion of the adult bees would still be working around the brood cells and therefore hidden from view. As part of the preliminary study, hives of moderate age from two suppliers were collected from tomato crops, subjected to the above assessment and then broken down in the laboratory to determine the proportion of the total that had been detected by that method. The results were reasonably consistent for each supplier. For hives from supplier A, an average of 76% of adult bees (range 72% - 81%) were recorded by the test method and, for supplier B, an average of 56% (range 48% - 61%) were recorded. The difference in 'recovery' from the two types of hives may be explained by the different brood chamber structures and type / quantity of wadding present. It was acknowledged that the technique described in the protocol would never give an absolute count of the number of adult bees present. However, it could provide a good indication of the level of activity and this was considered adequate for this survey.

The results from the 13 sites were examined individually to determine the point at which the Bta colonies began to go into decline after they had been placed in the tomato crop. The results were then collated so that any obvious differences between sites, bumblebee suppliers and tomato types could be identified. Finally, the combined results were summarised in charts to illustrate the overall duration of the Bta colonies.

## Objective 2. Bta biology & behaviour under different environmental conditions

Mini or standard Bta colonies were purchased from Agralan UK and on arrival at Warwick Crop Centre (WCC) were placed either within a glasshouse compartment or outside, according to the manufacturers recommendations. The experimental glasshouse ($44m^2$) contained tomato cv Piccolo grown as a long-season layered tomato crop according to commercial practice (see Objective 3 for further details). The 'outside' hives were placed in an apple orchard on the farm at WCC. Hives were housed within a corrugated plastic box and insulated with hay bales and tarpaulin for weather protection and also to replicate the underground nesting behavior of Bta (Figure 2). Hives were introduced a minimum of one week prior to observations and replaced every four weeks. On replacement, the hives were frozen and the colony destructively sampled and the number of bees, brood and eggs recorded.

**Figure 2. Location of hives in the experimental glasshouse (left) and outside (right)**

*Manual counts of Bta foraging trips*

‘Bee traffic’ was recorded as the rate at which bees entered and exited the hive per hour. This was done by counting the numbers of bees entering and exiting the hive every minute from sunrise to sunset. Data were then aggregated as numbers of bees entering / exiting per hour. Bee traffic for mini hives placed in the glasshouse and within the orchard was recorded on 14 August 2018 while activity from a standard hive placed within the orchard was recorded on 10 September 2018.

*Remote hive monitoring system*

An important task within this project was to develop a method of remotely monitoring Bta activity within hives that could be used in future studies. The work was done in collaboration with ‘Arnia Hive Monitors’ and involved adapting their honeybee remote monitoring system (HRMS) to work with bumblebee hives. The HRMS records changes in honeybee hive weight, brood temperature / humidity, outside temperature / rainfall and bee colony acoustic data (for details see https://www.arnia.co.uk). The latter provides an indication of whether the bees are foraging normally or performing other tasks such as ‘fanning’ to cool the brood. Our challenge was to modify the HRMS to collect similar data from the much smaller bumblebee colonies housed in considerably lighter structures of completely different design. Our goal was to develop a system that could be used to provide an insight into day / night activity cycles, including flight activity for bees entering / leaving the hive, as well as colony activity prior to the start of foraging. This would be used over the natural life of bumblebee colonies to show how that activity is affected by changes in the physical environment.

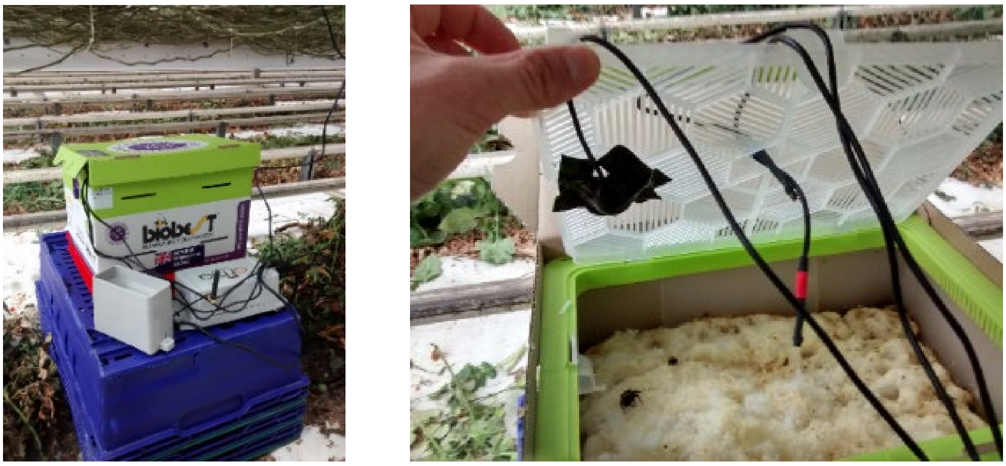


**Figure 3. The Arnia remote monitoring system adapted for a bumblebee hive**

Prototypes of the adapted HRMS were set up on the 13 August 2018 on bumblebee hives in the experimental glasshouse and in the WCC orchard and further monitors were set up in a commercial tomato crop at R & L Holt (Offenham, Worcestershire) on the 14 August 2018 (Figure 3). Data was recorded in real time and continuously uploaded to the 'Cloud' via a portal. All data was accessed remotely and interpreted from the researchers' own offices. This data was validated by comparison to the manual counts of bees leaving / returning to the hives.

## Objective 3. Flower development and pollen production in cv Piccolo

Tomato plants of cv Piccolo were produced by a commercial propagator on their own roots, and delivered to WCC on 4 June 2018. The first truss was evident on most plants upon delivery. The plants were grown-on in an experimental glasshouse compartment (6m x 6m) on 4 rows of rockwool slabs with each row consisting of 4 x 1.2m long rockwool slabs. Each slab carried 4 plants and so each row was made up of 16 plants and the 4 rows of slabs supported a total of 64 plants (128 heads) at a density of 4.68 heads per cropped m$^2$. Brian Moralee (Growing Manager, APS Produce), provided recommendations for producing 'generative' plants of cv Piccolo when planted at that time of year.

Two hives of bumblebees were introduced to the compartment on 20 June but it quickly became apparent that there were too many bumblebees in the relatively small compartment and so one hive was removed on 22 June. The remaining hive was removed on 4 July but was then returned for various periods. It was replaced by a new hive on 26 July but that too was removed later. Whenever bumblebees were absent, the trusses were 'buzzed' with an electric bee and pollination was assisted further by shaking the crop support wires.

A scale representing discrete and recognisable stages in the development of flowers of 'Piccolo' was devised (Figure 4). It comprised Stage 1 (flower buds visible); Stage 2 (petals visible); Stage 3 (bright yellow petals held at right angles to the pedicel); Stage 4 (flower open, petals reflexed); Stage 5 (flower closing, petals becoming pale); Stage 6 (flower closed, petals dying). This scale was then used to monitor the development of flowers along selected trusses initially for every hour from soon after dawn until dusk and later by records taken every 4 hours or so from 07:00 to 17:30 each day. The observations made in this way were confirmed using time-lapse photography.

We assessed pollen flow on the same flowers that were used for monitoring flower development and this was done by tapping the individual flowers ten times while holding them above a sheet of black card (Jacobson, 2007). The quantity of pollen released was then estimated on a scale ranging from 1 (none) to 3 (abundant). Unfortunately, the method proved relatively ineffective with cv Piccolo under our conditions and so we resorted to assessing the actual numbers of pollen grains that were produced by each flower. To do this, we selected one flower on each truss and began to take pollen samples from it once it had reached stage 4 (petals reflexed). In order to take a sample, the selected flower was held in a small PCR sealable vial and was vibrated with a mini-electric bee for five seconds to encourage its anthers to release their pollen. This was done at least four times a day for each flower and was repeated until the flower reached stage 5. The tubes with their pollen grains were then stored, by suspending them in 300µl of water, until there was time to count the grains on a haemocytometer slide under a microscope. The number of full grains present in particular cells on the slide was counted (empty grains and grain fragments were ignored) and two counts were made on each sample.

**Figure 4. The scale of stages of flower development in cv Piccolo**

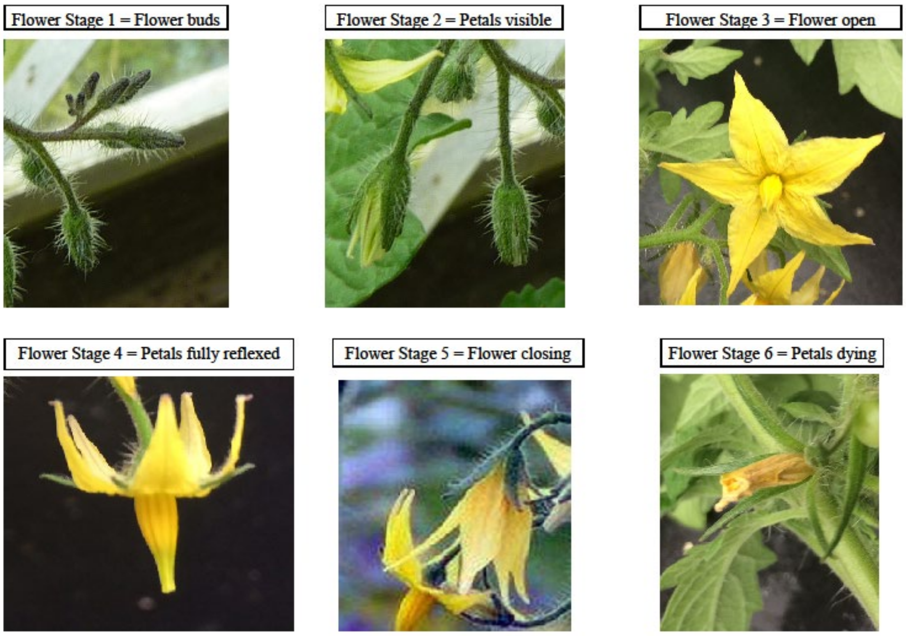



**Figure 4. The scale of stages of flower development in cv Piccolo**

In addition, the fruits produced on the first, second, third, fourth and sixth trusses of six plants were removed and, for each fruit, its position along the truss, its weight and diameter were recorded. Each fruit was then cut open, the seeds were separated from the pulp and other debris and all the seeds that were visible to the naked eye were counted and their number recorded.

## Results and Discussion

### Objective 1. Survey of bumblebee colony duration

The average numbers of adult bees recorded from 40 hives over a 12 week period at Site 1, using the method described in the protocol, are shown in Figure 5. Overall, the numbers of bees recorded started to decline within the first two weeks after the hives were placed in the tomato crop and continued to decline throughout the assessment period. Numbers varied between hives but only 3 of the 40 colonies (8%) bucked this overall trend; numbers in those hives increased over the first 2 weeks but went into decline thereafter.

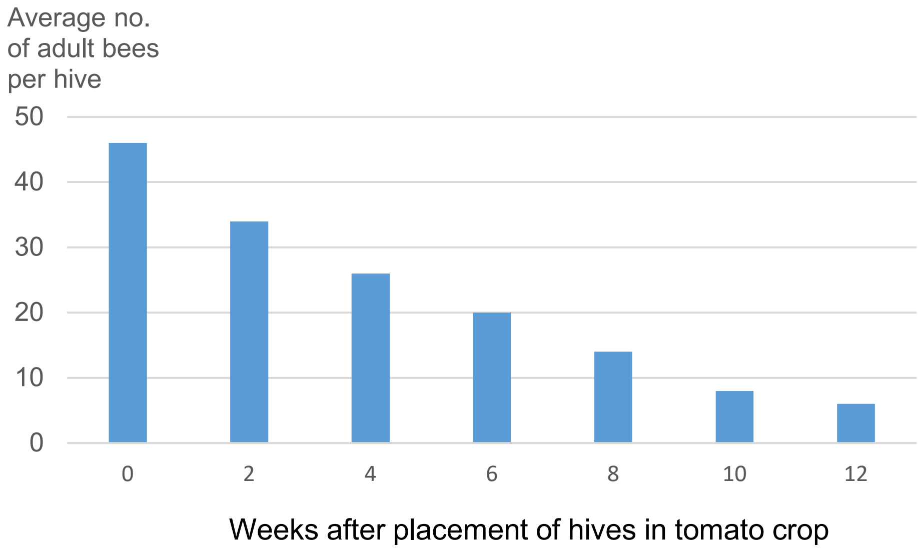



**Figure 5. Average numbers of adult bees recorded from 40 hives over a 12 week period at Site 1 (using the ‘activity index’).**

The average weight of the 40 hives over a 12 week period at Site 1 is shown in Figure 6. There was little variation in the weight of the hives upon delivery and it declined at a gradually reducing rate throughout the assessment period. The sugar solution incorporated beneath the brood chamber has the largest influence on the overall weight of the hive and the decline in weight reflects the amount of sugar solution that has been used. The lowest recorded weight from an individual ‘expired’ hive at this site is shown in Figure 6 and this provides an indication of the maximum use of sugar solution. A comparison of this with younger hives implies that sugar solution should not have been a limiting factor in colony duration throughout this assessment period. More detailed analysis of the available data may provide precise links between colony size and sugar usage should information about that relationship be required. However, such analysis was beyond the scope of this survey.

A summary of temperature and relative humidity (RH) data throughout the assessment period at Site 1, is shown in Table 2. This has been simplified to ease assimilation of the information and shows weekly averages of temperature and %RH as well as minimum and maximum records of both (commencing 06:00 hours Monday each week).

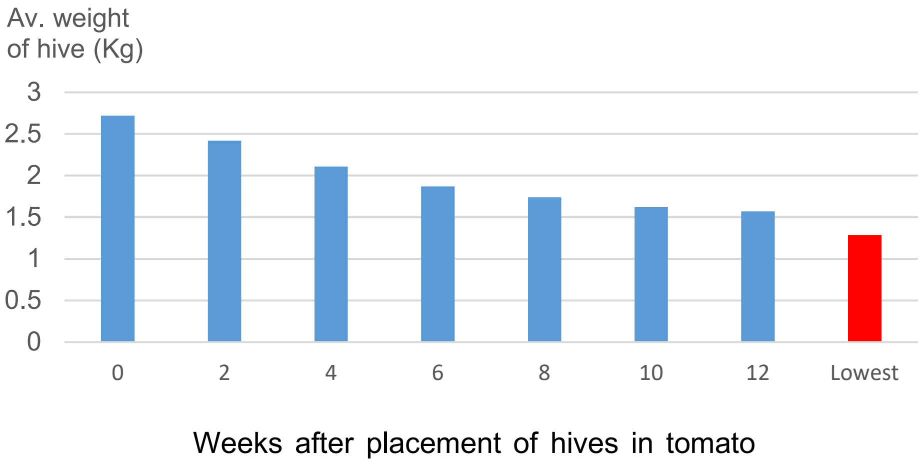



**Figure 6. Average weight of 40 hives over a 12 week period at Site 1. The lowest weight recorded from an individual 'expired' hive is shown to provide an indication of maximum use of sugar solution.**

The data collected from Site 2 were less well regimented than Site 1. New hives were delivered weekly to this site while assessments could only be done at 2 week intervals. As a consequence, some hives had already been in the crop for a week before being examined and this had a knock-on effect throughout the assessment period with greater variability in numbers of hives included at each data point than at Site 1. Nonetheless, the overall trend was similar to Site 1. Figure 7 shows the average numbers of adult bees recorded from 16 hives over an 8 week period at this site. The data collection was confounded from week 32 2018 by wasps invading the hives. This had become so serious by week 34 2018 that the assessments had to be abandoned thus reducing the numbers of records at the later data points. It is important to keep in mind the difficulties encountered at this site when examining the data in more detail. However, it was observed that numbers of adult bees were in decline within 2-3 weeks of placement in 64% of hives. Numbers in a further 14% of hives were in decline within 4 weeks of placement. The remainder were in decline within 5 weeks of placement.

**Table 2. Temperature and relative humidity readings at Site 1 throughout the assessment period (expressed as the weekly average, maximum and minimum from 06:00 hours each Monday morning).**

| Week number 2018 | Average temperature °C (Average %RH) | Minimum temperature °C (Minimum %RH) | Maximum temperature °C (Maximum %RH) |
|---|---|---|---|
| 26 | 21.2 (72) | 14.3 (42) | 27.8 (89) |
| 27 | 22.4 (66) | 15.1 (38) | 30.2 (86) |
| 28 | 22.3 (72) | 15.3 (51) | 29.8 (87) |
| 29 | 21.7 (73) | 15.3 (49) | 29.1 (85) |
| 30 | 21.9 (76) | 14.8 (44) | 29.3 (100) |
| 31 | 22.4 (75) | 14.9 (45) | 29.6 (88) |
| 32 | 21.8 (73) | 15.2 (53) | 27.6 (87) |
| 33 | 21.1 (74) | 14.8 (48) | 28.4 (88) |
| 34 | 20.3 (78) | 14.4 (60) | 26.3 (89) |
| 35 | 20.7 (72) | 14.6 (50) | 27.1 (90) |
| 36 | 19.5 (78) | 14.0 (61) | 26.3 (87) |
| 37 | 19.5 (77) | 14.5 (55) | 27.0 (89) |
| 38 | 19.6 (76) | 13.9 (53) | 26.4 (89) |
| Overall | 21.1 (74) | 14.7 (50) | 28.1 (89) |

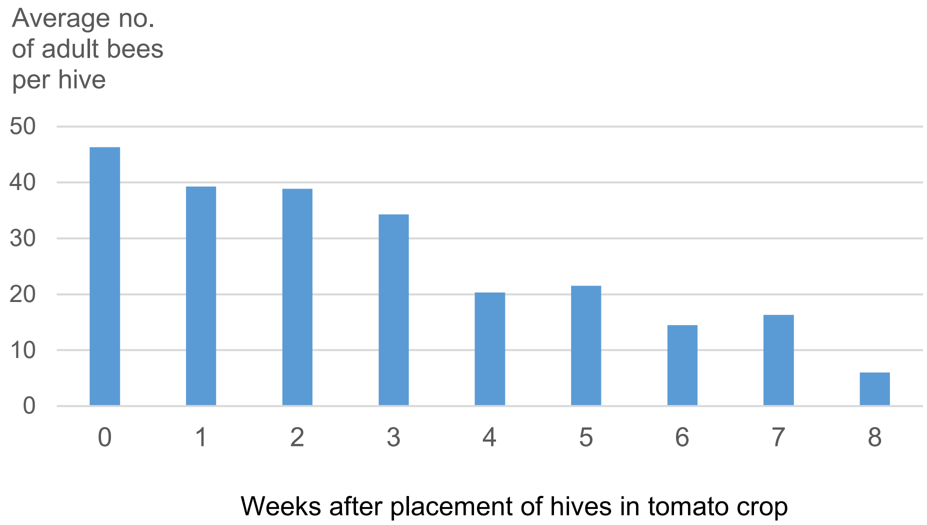



**Figure 7. Average numbers of adult bees recorded from 16 hives over an 8 week period at Site 2.**

The invasion by wasps also affected the weight of hives. Towards the end of the assessment period the rate of decline in the weight of hives doubled – presumably due to wasps stealing sugar solution. As a consequence, the data from the worst affected hives has not been included in this report. The average weight of the remaining 14 hives over a 5 week period at Site 2 is shown in Figure 8, as is the lowest recorded weight from an individual 'expired' hive. Comparison of the latter with younger hives once again suggests that sugar solution should not have been a limiting factor in colony duration. Due to the confounding factors described above, it is unlikely that there would be anything to be gained from further analysis of data from Site 2.

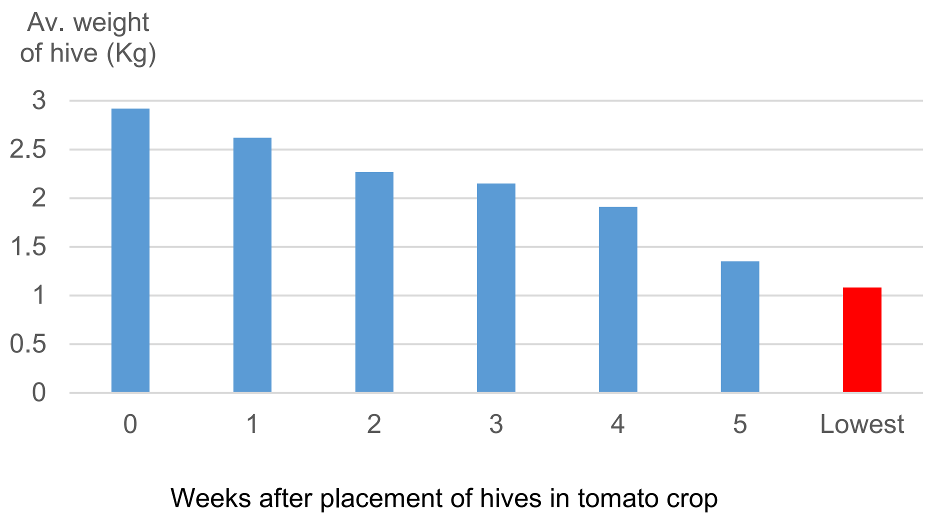



**Figure 8. Average weight of 14 hives over a 5 week period at Site 2. The lowest weight recorded from an individual 'expired' hive is shown to provide an indication of maximum use of sugar solution.**

The mean activity indices of Bta colonies at each of the 12 sites over an 8 week period from the time of hive delivery to the nursery is shown in Table 3. Of the 161 hives included in the national survey, only 12 (7%) showed an increase in the activity index after deliver to the nursery. In most cases, this increase was not sustained beyond one assessment. If this is expressed in terms of the number of individual colony assessments completed during the study, then there was an increase in activity index following only 2% of the 777 assessments. The results were broadly comparable for all bumblebee suppliers, geographical locations and tomato types. It was therefore appropriate to combine the data from all sites to provide an overall national summary (Figure 9). This shows a steady decline in activity index throughout that 8 week period.

**Table 3. Mean activity index of Bta colonies at each site at five intervals after delivery of hives to the 12 UK tomato production sites.**

| Site | Mean activity index at the following weeks after delivery: | | | | |
|---|---|---|---|---|---|
| | 0 | 0-2 | 2-4 | 4-6 | 6-8 |
| 1 | 2.9 | 2.5 | 2.1 | 1.7 | 1.4 |
| 2 | 2.9 | 2.9 | 2.3 | 1.8 | 1.3 |
| 3 | 3.0 | 2.8 | 2.0 | 1.0 | 1.0 |
| 4 | 3.0 | 3.0 | 2.3 | 2.0 | 0.3 |
| 5 | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 |
| 6 | 3.0 | 3.0 | 2.3 | 2.1 | 1.4 |
| 7 | 3.0 | 3.0 | 2.0 | 2.0 | 2.0 |
| 9 | 2.6 | 1.8 | 1.3 | 1.1 | - |
| 10 | 2.3 | 2.0 | 1.4 | 1.4 | 1.3 |
| 11 | 3.0 | 1.6 | 1.1 | 1.4 | - |
| 12 | 3.0 | 1.6 | 1.5 | 1.4 | 1.1 |
| 13 | 2.8 | 1.5 | 1.1 | 0.9 | - |
| Average | 2.8 | 2.3 | 1.7 | 1.5 | 1.2 |

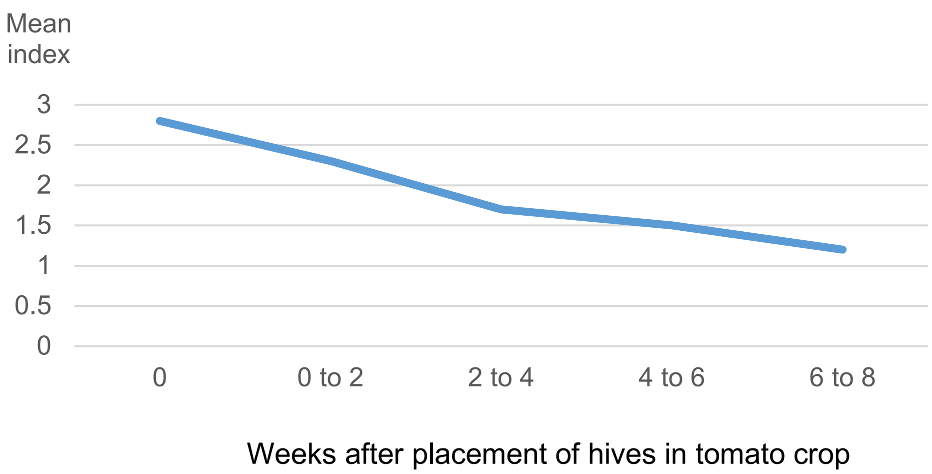



**Figure 9. The overall mean of activity indices of all Bta colonies at all 12 tomato production sites at 5 intervals following delivery of hives to those sites**

In addition to the activity index data over time, it is useful to investigate when the individual colonies started to go into decline. Table 4 shows the number (and percentage of total) of colonies at each site that started to go into decline 0-2, 2-4, 4-6 and 6-8 weeks after delivery to the nursery. There was some variability between sites but without any obvious pattern. Overall, 62% of colonies went into decline during the first two weeks of being placed in the tomato crop and a further 28% had gone into decline by the fourth week. For ease of interpretation, the overall results are illustrated graphically in Figure 10.

**Table 4. Numbers of colonies that began to go into decline during four periods after delivery to the tomato production sites; *i.e.* 0-2, 2-4, 4-6 and 6-8 weeks.**

| Site | Number of hives | Mean index upon delivery | Numbers (and percentages) of colonies that started to decline after the following periods in the tomato crop: | | | |
|---|---|---|---|---|---|---|
| | | | 0-2 | 2-4 | 4-6 | 6-8 |
| 1 | 40 | 2.9 | 28 (70%) | 9 (23%) | 3 (7%) | - |
| 2 | 16 | 2.9 | 5 (31%) | 8 (50%) | 3 (19%) | - |
| 3 | 4 | 3.0 | 1 (25%) | 3 (75%) | - | - |
| 4 | 3 | 3.0 | 0 (0%) | 2 (66%) | 1 (33%) | - |
| 5 | 4 | 2.0 | 0 (0%) | 4 (100%) | - | - |
| 6 | 10 | 3.0 | 0 (0%) | 7 (70%) | 2 (20%) | 1 (10%) |
| 7 | 4 | 3.0 | 0 (0%) | 4 (100%) | - | - |
| 9 | 18 | 2.6 | 18 (100%) | - | - | - |
| 10 | 16 | 2.3 | 4 (25%) | 7 (44%) | 1 (6%) | 4 (25%) |
| 11 | 10 | 3.0 | 9 (90%) | 1 (10%) | - | - |
| 12 | 10 | 3.0 | 9 (90%) | 0 (0%) | 1 (10%) | - |
| 13 | 26 | 2.8 | 26 (100%) | - | - | - |
| All sites | 161 | 2.8 | 100 (62%) | 45 (28%) | 11 (7%) | 5 (3%) |

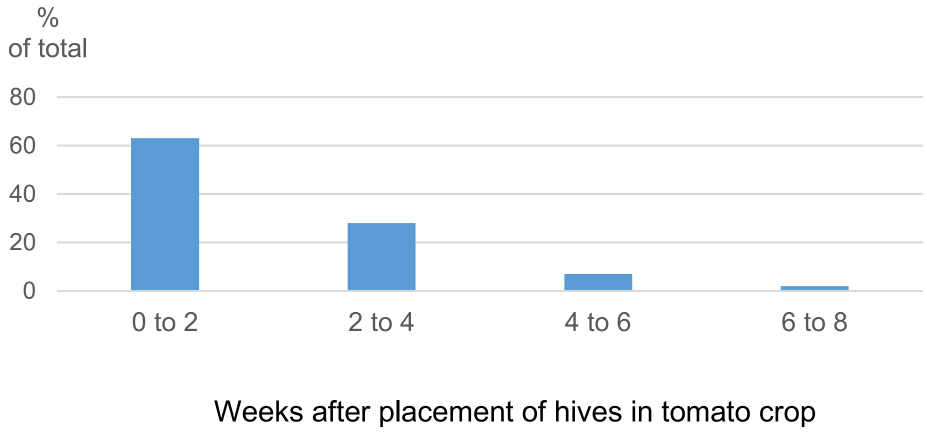



**Figure 10. The time after delivery that 161 Bta colonies, across 12 UK tomato production sites, went into decline after delivery to those sites (expressed as the percentage of the total at 0-2, 2-4, 4-6 and 6-8 weeks post-delivery).**

## Objective 2. Bta biology & behaviour under different environmental conditions

*Manual counts of Bta foraging trips*

Adult Bta activity was observed between dawn (05:10h) and sunset (20:32h) on 14 August 2018. Light levels ranged from 0 to 590 w/m$^2$ (average 183 w/m$^2$) peaking between 11:00h and 14:00h (Figure 11). Temperature ranged from 17.3$^O$C to 26.9$^O$C (Average 21.2$^O$C) in the glasshouse and 14.6$^O$C to 23.7$^O$C (Average 18.4$^O$C) in the orchard (Figure 11) with peaks between 13:00h and 14:00h.

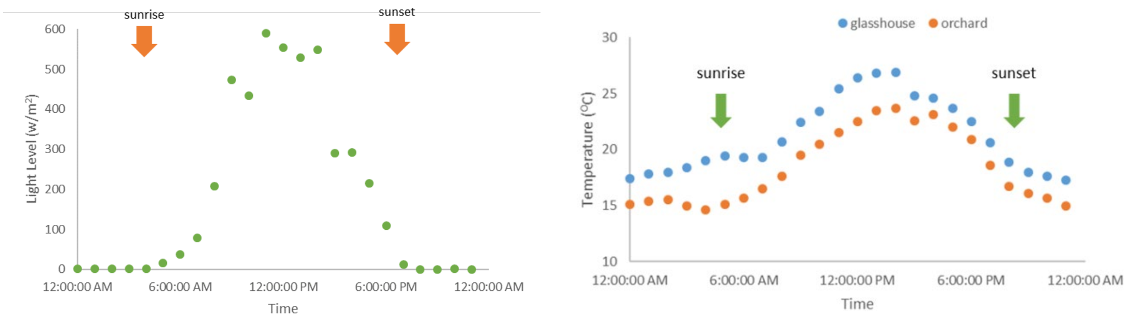
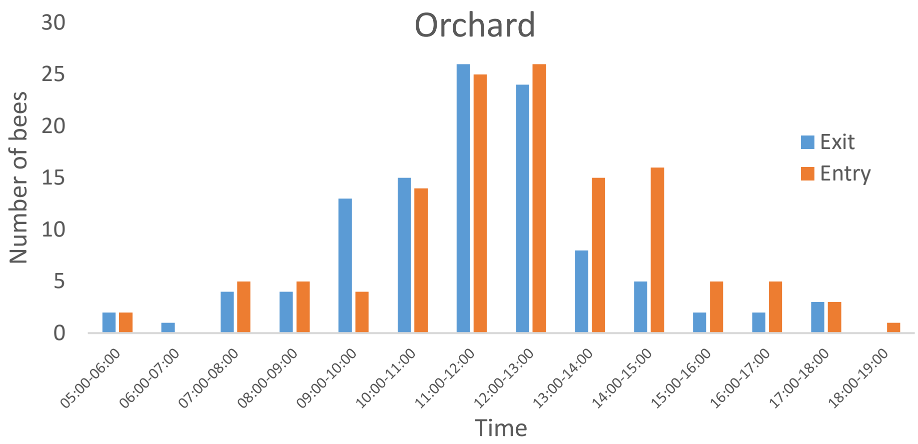



**Figure 11. Light levels (w/m$^2$) and air temperature (°C) within the glasshouse and orchard 14th August 2018**

On the 14 August, bee flights began between dawn and sunrise (05.49h) and peaked when the sun was at its highest (13:11pm) (Figure12). No flights were observed within 2 hours of sunset. The first exit was at 05:45h from the hive in the glasshouse and lasted c45 minutes, while the first exit in the orchard was 05:30 and lasted c9 minutes. Peak bee activity occurred between 11:00h and 12:00h in the glasshouse and between 11:00h and 13:00h in the orchard. However, activity was considerably greater in the orchard than in the glasshouse, with 26 bees exiting and 24 bees entering the hive in the orchard between 11:00h and 13:00h but only 6 bees exiting and 5 bees entering the hive in the glasshouse during the same time period. The last flights from the orchard and glasshouse hives were at 17:40h and 18:21h respectively. No activity was observed after 18.45h from either of the hives. Pollen loads were not recorded, however bees in the glasshouse were rarely seen with full pollen sacs.



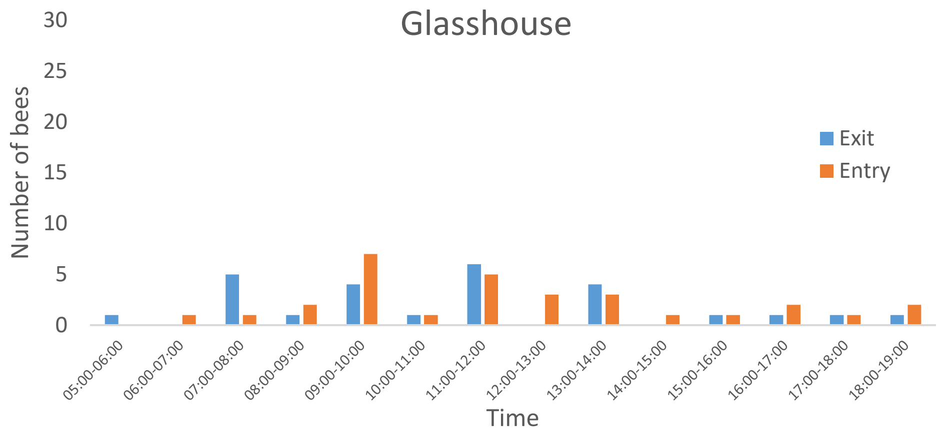




**Figure 12. Number of bees entering and exiting the hives positioned in the orchard and the glasshouse on 14 August 2018**

On the 10 September, adult Bta activity was observed between dawn (05:59h) and sunset (19:32h). Light levels ranged from 0 to 531 w/m$^2$ (average 148 w/m$^2$) peaking at noon (Figure 13). Temperature ranged from 11.2$^O$C to 18.8$^O$C (Average 15.4$^O$C) peaking between 14:00h and 15:00h (Figure 13).

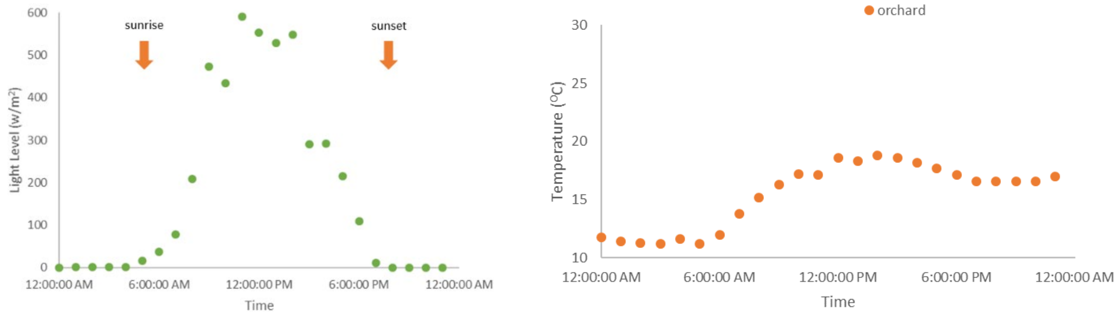



**Figure 13. Light levels (w/m$^2$) and air temperature (°C) in the orchard on 10 September 2018**

On the 10 September, bee flights began between dawn and sunrise (06.33h) and peaked when the sun was at its highest (13:03pm) (Figure 14). No flights were observed within 1 hour of sunset. The first exit was at 06:48h and the flight lasted for c12 minutes. Flight activity increased from 08:00h and peaked between 13:00h and 14:00h with 84 bees exiting and 24 bees entering the hive between 11:00h and 13:00h. The last flight was at 18:18h and no activity was observed after 6.20h.

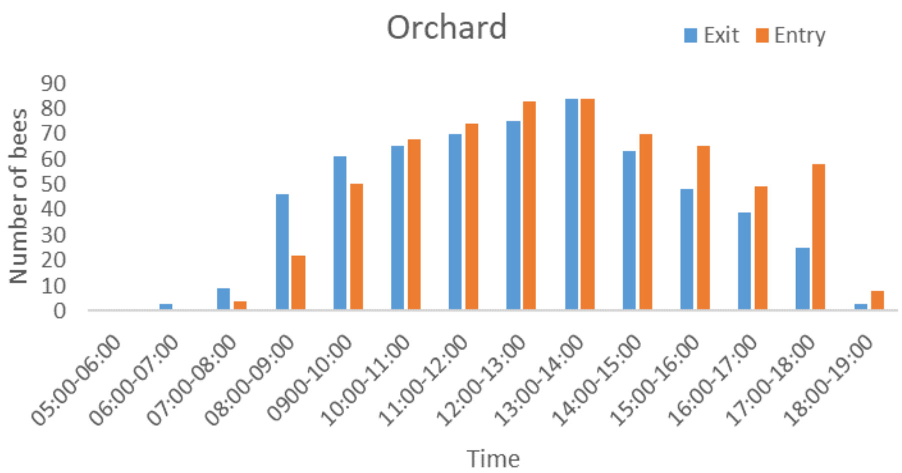



**Figure 14. Number of bees entering and exiting the hive within the orchard on 10 September 2018**

*Destructive colony assessments*

The number of adult bees found in the mini hives four weeks after delivery ranged from 32 to 45 (Table 5). All colonies contained some brood but only one colony (glasshouse, August) contained any eggs. There was no correlation between location of hive and colony size. The standard hive consisted of 93 adult bees, 14 pupae, 6 larvae and 8 eggs. Considerable variation in bumblebee size was observed in most colonies, with bees ranging in size from 0.9cm to 2.4cm (Figure 15).

**Table 5. Number of adult bees, pupae, larvae and eggs recovered from colonies four weeks after delivery and positioned within the glasshouse or orchard**

| | | | Total number of | | | |
|---|---|---|---|---|---|---|
| **Hive** | **Location** | **Time** | **Adult** | **Pupae** | **Larvae** | **Eggs** |
| Mini | glasshouse | July | 32 | 7 | 2 | 0 |
| Mini | orchard | July | 42 | 0 | 18 | 0 |
| Mini | glasshouse | August | 45 | 5 | 35 | 18 |
| Mini | orchard | August | 34 | 40 | 22 | 0 |
| Standard | orchard | September | 93 | 14 | 6 | 8 |


**Figure 15. Size range of adult Bta in a commercial hive**

*Remote hive monitoring system*

The Arnia RHMS successfully generated data from Bta hives placed in the orchard at WCC and within glasshouses both at WCC and the commercial tomato nursery. Data from the monitors was uploaded to the ‘Cloud’ twice a day and could be accessed easily using a PC, tablet or smartphone. A number of alterations were done in collaboration with the team at Arnia Hive Monitors to improve data quality. These included recalibrations of the scale load cells to generate improved measurements of hive weight, as well as work to determine the best positions for the within-hive temperature, humidity and acoustic sensors. Small holes had to be cut into the hives to accommodate the sensors, but this was easy to do and did not disturb the bees to an unmanageable extent.

Over the course of the project, data from Arnia monitors were obtained from: i) two mini-hives set up in the glasshouse and apple orchard at WCC (August 2018), ii) two standard hives in the commercial production greenhouse (August-September 2018) and iii) a standard hive placed in the orchard at WCC (September 2018). All hives showed a diurnal pattern in acoustic data, with noise levels for flight activity peaking in the day and then declining to a trough overnight (*e.g.* Figure 16 for data on mean flight noise and total bee activity from the hive in the orchard in September). This pattern appeared to reflect the daily bee flight pattern record from manual counts of bee traffic (Figures 17 & 18). Hive weights declined during the monitoring period, which presumably reflected consumption of sugar within the feed reservoir. The Arnia load cell used with the standard hive in the orchard also showed a diurnal change in weight (Figure 17) which is probably caused by bees leaving the hive during the day to forage. The hives positioned within glasshouses did not show this diurnal change in weight (data not shown), which may be a result of poor bee flight activity from hives within the glasshouse or a lack of sensitivity for load cells used in the glasshouse. The hives in the orchard were placed on a concrete slab and it is possible that this provided a more stable base for the load cells.

For hives placed outside, brood temperature was relatively stable compared to outside air temperature. For the mini-hive in the orchard, brood temperature ranged from 17.8°C to 25.6°C (mean = 21.8°C) whereas air temperature ranged from 9.9°C to 28.9°C (mean = 19.2°C). As expected, mean temperatures in glasshouses were higher. Brood temperature for the mini-hive in the glasshouse ranged from 20.9 °C to 29.9 °C (mean = 26.7 °C) while air temperature ranged from 16.8°C to 26.1°C (mean = 21.5°C). Brood temperature for the standard hive placed outside in the orchard in September 2018 was also relatively stable compared to ambient air temperature. For example, on 10 September 2018 (= the day that manual bee traffic counts were done), brood temperature ranged from 26 °C to 29.5 °C (mean = 27.5 °C) while air temperature ranged from 8.9°C to 22.1°C (mean = 15.7°C) (Figure 18). The mean brood temperatures for the two standard hives in the commercial nursery were 23.7 °C and 22 °C respectively and fluctuated from the mean by +/- 5 °C. None of the hives monitored in the project were subject to the high temperatures experienced during the peak of the 2018 summer and it remains to be seen whether very high ambient temperatures within a commercial glasshouse result in detrimental elevations in brood temperatures.

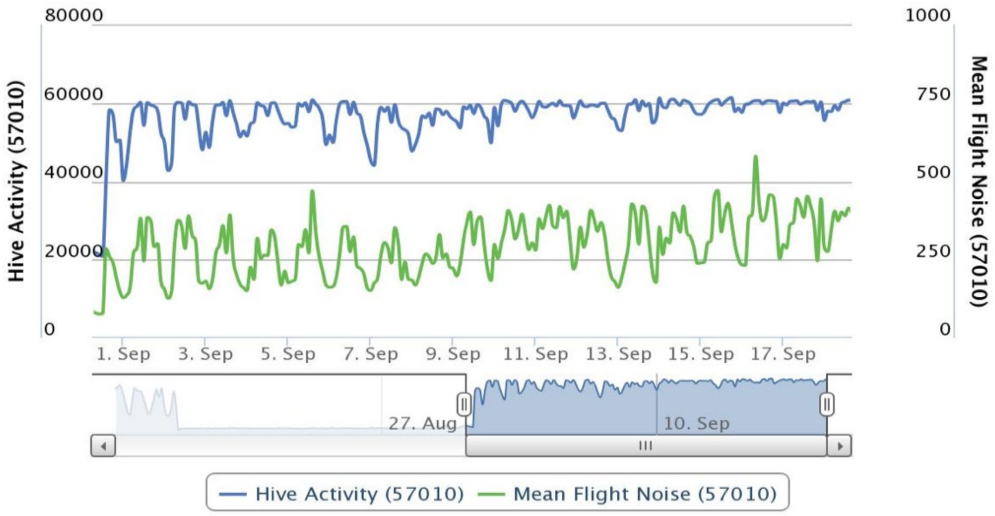



**Figure 16. Diurnal fluctuation in acoustic data (hive activity and mean flight noise) recorded in a standard bumble bee hive placed within the apple orchard at WCC in September 2018.**


**Figure 17. Weight of the standard Bta hive in the WCC orchard in September 2018.**

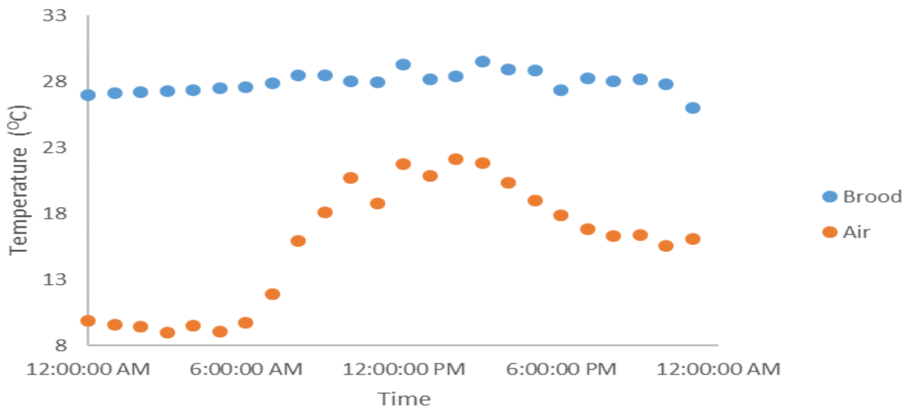



**Figure 18. Brood temperature and outside air temperature recorded with the Arnia monitor within the orchard hive on 10 September 2018**

### Objective 3. Flower development and pollen production in 'Piccolo'

The observations of flower development confirmed that, as expected, successive flowers opened in sequence along a truss but they also demonstrated that individual flowers were usually open on at least two successive days. The development of a typical individual flower is shown in Figure 19 which shows the data collected from the sixth flower on the 13$^{th}$ truss. Although Figure 19 suggests that flowers that reached stage 4 (petals fully reflexed) subsequently moved straight on to stage 5 (flower closing), time-lapse photography revealed that the petals of flowers that reached stage 4 on one day, almost always closed up in the late afternoon / early evening before the onset of darkness and then re-opened to stage 4 again the following morning. The recorded data and the time-lapse sequences showed that there could be as many as four flowers open on each truss at most times of day and, sometimes as many as five flowers were open at the same time on any individual truss.

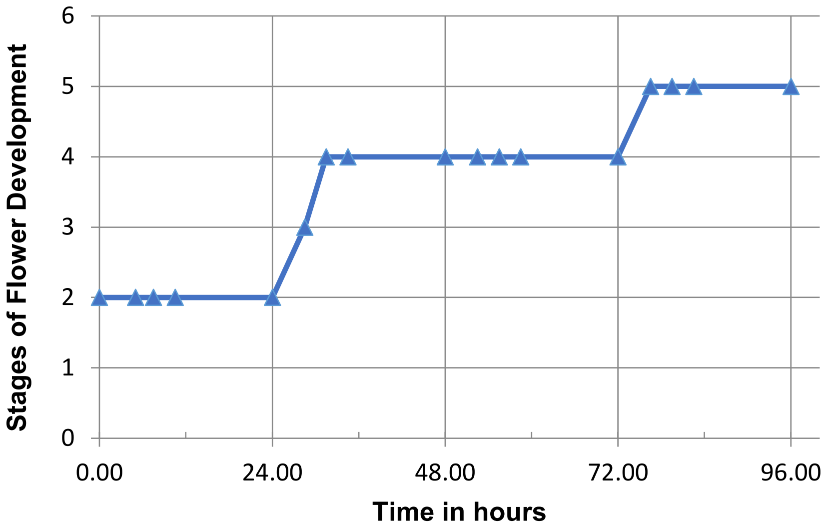



**Figure 19. The typical course of development followed by flowers of cv Piccolo. In this example, the flower was the sixth flower on the 13th truss.**

In order to link flower development to the flow of pollen, we used the method of tapping each flower while holding it over a black card (Jacobson, 2007). Although this approach had worked successfully on other cultivars it did not work well under our conditions as we rarely obtained abundant quantities of pollen. In general, however, although some pollen was released from the anthers of flowers at stage 2, pollen was more likely to be released from the anthers of flowers at stage 4 (*i.e.* petals fully reflexed).

It was beyond the scope of this project to investigate whether the apparent failure to release large quantities of pollen was a characteristic of the cv Piccolo, or was due to the high temperatures experienced that summer, or to some feature of the glasshouse compartment, such as its fan-assisted ventilation. Instead, we estimated pollen production directly by counting how many pollen grains were released from the anthers of each of the flowers on selected trusses. The results are shown in Table 6 from which it appeared that flowers sampled in late August produced an average of 22,872 pollen grains per flower while those sampled in late September produced 44,246 pollen grains per flower. In addition, the variability in pollen production per flower was much greater in the flowers sampled in August than in those sampled in September.

**Table 6. Pollen Counts**

| 20-24 August | | | 25-28 September | | |
|---|---|---|---|---|---|
| Plant | Number of grains counted | Total per flower | Plant | Number of grains counted | Total per flower |
| 1 | 2326 | 13956 | 1 | 9542 | 57252 |
| 2 | 3034 | 18204 | 2 | 8672 | 52032 |
| 3 | 65.5 | 393 | 3 | 4413 | 26478 |
| 4 | 3371 | 20226 | 4 | 12107 | 72642 |
| 5 | 13271 | 79626 | 5 | 9948 | 59688 |
| 6 | 4545 | 27270 | 6 | 5711 | 34266 |
| 7 | 3521 | 21126 | 7 | 5368 | 32208 |
| 8 | 594 | 3564 | 8 | 3026 | 18156 |
| 9 | 2543 | 15258 | 9 | 10302 | 61812 |
| 10 | 4353 | 26118 | 10 | 6766 | 40596 |
| 11 | 6628 | 39768 | 11 | 5101 | 30606 |
| 12 | 1493 | 8958 | 12 | 7536 | 45216 |
| Average | 3812.0 | 22872.3 | Average | 7374.3 | 44246.0 |

As the amount of pollen released from each sampled flower was counted on four occasions per day we were able to note the time that most pollen was released from each flower, *i.e.* peak pollen release (Table 7). The data showed that the peak of pollen release generally occurred after mid-day and that the overall average of the samples taken in August and in September was 13:03h.

According to the published literature, the period during which pollen grains are being formed is very sensitive to disruption by high temperature as is whether the pollen is released or retained in the anther together with the viability of the pollen that is released (*eg.* Sato and Peet, 2005). According to an earlier paper (Sato, Peet & Thomas, 2000), pollen formation occurs in the period from about 10 days before anthesis up to 7 days before anthesis, while high temperature by day during the period from 15 days before anthesis up to 7 days before anthesis influences whether the pollen is released from the anthers. The average day temperatures between 12:00h and 15:00h over these periods in our experiment are shown in Table 8 both for the samples of flowers taken in August and those taken in September. We also show the average day temperatures between 12:00h and 15:00h for the period from 18 to 13 days before anthesis, when day temperatures were particularly high in August 2018, *i.e.* 2 to 7 August as compared to 7 to 12 September.

**Table 7. Time of peak pollen release**

| Flower No. | August 21-23 | September 25 - 27 |
|---|---|---|
| 1 | 13:30 | 11:30 |
| 2 | 11:00 | 14:30 |
| 3 | 16:30 | 11:30 |
| 4 | 13:30 | 13:30 |
| 5 | 16:45 | 14:30 |
| 6 | 13:45 | 14:30 |
| 7 | 09:00 | 11:30 |
| 8 | 13:45 | 14:30 |
| 9 | 11:30 | 11:30 |
| 10 | 09:00 | 11:30 |
| 11 | 11:30 | 16:30 |
| 12 | 11:30 | 16:30 |
| Average | 12:36 | 13:30 |

**Table 8. The average day temperatures prior to anthesis**

| Time | Period | Period | Difference |
|---|---|---|---|
| | Aug 5 to 13 | Sep 11 to 17 | |
| 12:00 to 15:00 | 24.5°C | 21.6°C | 2.9°C |
| | Aug 2 to 7 | Sep 7 to 12 | |
| 12:00 to 15:00 | 29.4°C | 21.1°C | 8.3°C |

Clearly, the average day temperature between 12:00 and 15:00 was hotter by nearly 3°C in the period from 15 days before anthesis to 7 days before anthesis and the average difference was more than 8°C in the period from 18 to 13 days before anthesis. Although the highest temperature we encountered was much lower than the day temperature of 32°C employed by Sato, Peet and Thomas (2000), it nevertheless seems likely that more pollen was produced in September than in August because of the lower day-time air temperature in September. It is also important to know how much pollen is released from the flower's anthers and when it is released. Our observations with the Jacobson (2007) method of monitoring pollen release suggest that the release of pollen was a problem with cv Piccolo especially in the day temperatures encountered in the summer of 2018.

Seed counts (Table 9) showed that each fruit of 'Piccolo' contained an average of 76 seeds while the greatest number of seeds in any single fruit was 120. Thus, it appears that no more than 120 viable pollen grains are normally needed in order to fertilise all the ovules in the ovary of a cv Piccolo flower and yet each flower is capable of producing at least 20,000 pollen grains. For comparison, a classic round tomato (cv Sunbeam) from R&L Holt's nursery in Offenham contained an average of 155 seeds per fruit and its flowers too produced many more pollen grains than were required to produce these fruits. However, as we stressed earlier, as well as the number of pollen grains produced by the anthers, the number released from them and the number that are viable are also features of great importance.

**Table 9. Fruit characteristics**

| | Fruit diameter (mm) | Fruit weight (gm) | Number of seeds per fruit |
|---|---|---|---|
| Truss 1 | 25.86 | 10.09 | 70.40 |
| Truss 2 | 23.70 | 8.56 | 75.26 |
| Truss 3 | 22.06 | 7.58 | 76.84 |
| Truss 4 | - | 8.09 | 80.17 |
| Truss 6 | 27.47 | 12.18 | 77.45 |
| Mean | 24.77 | 9.30 | 76.03 |

At some times during the experiment, as many as one third of all the trusses were split trusses which, we are assured, is not unusual for cv Piccolo grown at that time of year.

Our findings may be summarised as follows:

- Each flower was usually open on two successive days although it usually released most of its pollen on the first day. The peak of pollen release usually occurred between 12:30h and 13:30h each afternoon but, for some flowers the peak occurred much earlier and for others it occurred much later.
- The tomato flower is normally self-pollinated and it was evident that 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.

- Although it appeared that each flower produced and released a vast excess of pollen when the flower was shaken with a mini electric bee, further work is required to establish that similar amounts would be released by the actions of bumblebees or by the actions of growers. It is also important to establish the proportion of the pollen grains that are viable and able to germinate.
- Our observations suggest that pollen production and pollen release are inhibited by high day temperatures of $29^0$C or more which is also the conclusion reached in the published literature. High day temperatures may also have contributed to the variability in pollen production by different flowers, as that was more noticeable in August than in September, and the high temperature may also have affected pollen viability.
- Unfortunately there were no opportunities to manipulate the cropping environment in a controlled manner in this experiment because, after the crop had been established in June, there followed a very long period of unusually high ambient temperatures that lasted throughout much of the summer.

## Conclusions

### Objective 1. Survey of bumblebee colony duration

- 62% of Bta colonies went into decline within 2 weeks of being placed in a tomato crop. This had increased to 90% within 4 weeks of delivery. There were no consistent differences between bumblebee supplier, geographical location or tomato type. This rapid decline was in contrast to our expectations based on previous studies with the non-native bumblebees (see Figure 1).
- There were variations in numbers of adult bumblebees in hives upon delivery from all suppliers but this was also known to be the case with the non-native bumblebees. The Bta hives generally contained good active colonies upon delivery and we would have expected these to continue to increase in size before completing their cycle.
- The only two common factors across all the sites appeared to be Bta bumblebees and tomato crops; thus suggesting that the former either do not like the tomato growing environment or the plants do not provide a satisfactory food source.
- We must stress that these results relate to the duration of the colonies and do not necessarily mean that the bumblebees failed to provide good fruit set during the monitored period. However, the results do begin to explain why more Bta hives are required than was the case with Btt/Btd and why some growers have obtained improved results by moving to a weekly hive input schedule.

### Objective 2. Bta biology & behaviour under different environmental conditions

- Bees were generally active over a 12 hour period of the day. Activity followed a similar pattern regardless of hive location, temperature or time of year, with the first activity seen just after sunrise, rising to a peak in activity between 11:00h and 14:00h followed by a fall in activity around 18:00h.
- Often two short peaks of daily activity were recorded in the glasshouse colonies but only one, more prolonged, peak observed in the orchard colonies.
- Activity in the orchard colonies was considerably higher than that observed in the glasshouse tomato crops.
- Brood and glasshouse temperatures never exceeded 30°C and there was no evidence that the activity recorded was correlated with temperature. Available literature suggests that *B. terrestris* will generally only cease to forage at temperatures above 32°C (Kwon and Saeed, 2003).

- Arnia monitors not only provided continual and more detailed information on hive activity than the time consuming, labour intensive manual counts but they also provided in depth information on the hive environment / health and will provide a valuable tool for future studies.

**Objective 3. Flower development and pollen production in cv Piccolo**

- Cv Piccolo is usually sold 'on the vine' and so the failure to set fruit by one or more flowers on a truss poses problems for the marketing of that truss. This failure to set fruit may occur with just one flower or it may afflict a number of flowers in the same section of the truss. In essence, the failure to set fruit seems to be due to a failure to transfer enough viable pollen from the anthers to the stigma of the same flower.
- Our observations show that the flowers of cv Piccolo normally vary in the amount of pollen they produce and the time of day when they release it. It is also evident that high day temperatures interfere with the production of pollen and with its release from the anthers and that the consistently high day temperatures in the summer of 2018 also increased the variability in pollen production between different flowers.
- In addition, there is uncertainty about the proportion of the pollen that is viable; *i.e.* pollen that can germinate on a stigma and produce a pollen tube that will penetrate the style and reach an ovule within the ovary.
- Our observations suggest that the bumblebees should visit flowers on the day that the flowers first open and, preferably, around 13:00h which is the time that most flowers release their largest numbers of pollen grains. However, some flowers release most pollen as early as 09:00h and some as late as 16:30h and so some bumblebees must be foraging at all times of day.
- Our observations also show that flowers produce less pollen when the day temperature increases, and it may be that bumblebees are then deterred from visiting flowers of cv Piccolo as the 'rewards' in terms of pollen availability are greatly reduced. It is also possible that the bumblebees are deterred from visiting cv Piccolo flowers because of some other factor that is unique to this cultivar, such as the production of a volatile chemical. Reports from some growers support this in general for they suggest that, when given a choice, the bumblebees prefer the flowers of other cultivars.
- As up to five flowers may be open on each truss at any one time it is possible that this may cause bumblebees to fail to visit each flower on its first day of opening and at the time when it is freely releasing pollen. It is evident that careful observation of when and how bumblebees visit trusses and whether they visit all or only some of the flowers is required.

- Although various factors could contribute to the failure to transfer enough viable pollen to the stigmas of tomato flowers, a major cause could be that the bumblebees fail to visit all of the flowers at a time when they are each producing adequate quantities of viable pollen and may even be a failure of the bumblebees to release the pollen from the flower's anthers.

## Knowledge and Technology Transfer

- Chandler & Jacobson (2018). Participation in bumblebee debate with growers and supply company, Tomato Study Group, Glen Avon Growers, Cottingham, East Yorkshire, 21 August 2018
- Chandler & Jacobson (2018). Presentation at the British Tomato Conference, Kenilworth, 27 September 2018.
- Jacobson (2018). Articles in TGA Newsletters in:
    - June 2018
    - August 2018
- Jacobson (2018). 'Buzzed Off'. Article in 'The Grower' (Journal of AHDB Horticulture). Issue 239, April / May 2018
- Jacobson (2018). Article for 'The Grower' (Journal of AHDB Horticulture) to be delivered on 3 December 2018 for February 2019 issue.
- Jacobson (2018). Reports to TGA Technical Committee meetings:
    - Via conference call, 4 April 2018
    - Stoneleigh, 6 June 2018
    - Kenilworth, 28 September 2018
    - Stoneleigh, 5 December 2018 (requested by TGA TC)
- Jacobson (2018). Presentation to the Tomato Study Group, Red roofs Nursery, Cottingham, East Yorkshire, 15 May 2018

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