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TOMATO GROWERS' ASSOCIATION

ANNUAL REPORT

To:

AHDB Horticulture

Stoneleigh Park, Kenilworth

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

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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
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Report:	Annual report, 15 February 2020
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Date project commenced:	1 January 2019
Date project completed:	31 December 2020

DISCLAIMER

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

- Bta produced larger colonies in an outdoor habitat than in glasshouse tomato crops.
- Bta flight activity in an outdoor habitat was substantially greater than in tomato crops.
- Bta flights in tomato crops coincided with pollen availability.
- Methods have been developed to further investigate Bta flight activity, tomato flower development, pollen flow and pollen viability.

Background

British tomato growers had successfully pollinated their crops with two non-native sub-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. NE suggested that non-native bumblebees could escape from glasshouses and hybridise with the British native sub-species, *B. terrestris audax* (Bta), leading to the local extinction of Bta. As a consequence of the NE decision, growers had to switch to Bta in the 2015/16 growing season. However, Bta failed to provide the reliable and maintenance-free pollination experience to which the industry had become accustomed. This should not have been a surprise because at least one of the producers, Brinkman Bunting Bumblebees BV, had already tested and dismissed Bta due to inferior performance during the 1980s.

British tomato growers are keen to use Bta if this can be done without significant economic loss and they have instigated several studies since 2015 in an attempt to improve the situation. The first 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 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 Bta to be complex with significant differences between populations from different parts of the British Isles. This information, along with known hybridisation among European mainland sub-species, raises questions about the validity of the current classification of *B. terrestris* sub-species.

In 2017, the TGA Technical Committee (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. The survey was repeated in 2019. It identified a marginal improvement in growers' perception of the performance of Bta, which could perhaps be attributed to improved Bta breeding stock and / or improved in-crop management of Bta. Nonetheless, growers still considered Btt/Btd to be substantially superior to Bta.

In 2018, the TGA TC organised a short (6 months) AHDB-funded practical project to begin to investigate factors raised by tomato growers in three key subject areas. First, the team discovered that most Bta colonies went into decline soon after placement in tomato crops, which was in stark contrast to previous experience with Btt/Btd colonies. Second, a study of traffic from Bta hives strongly indicated that there was considerably less flight activity in glasshouse tomato crops than outdoors. Finally, a preliminary study to investigate flower development and pollen production in cv Piccolo provided a foundation for more detailed experimentation.

Based on the results from all the above studies, the TGA TC organised the present AHDB-funded project to further investigate the following important factors:

- Activity of Bta in commercial tomato crops in the UK.
- Effects of high temperatures on within-hive behaviour of Bta and Btt/Btd.
- Effects of high temperatures on pollen production / viability with emphasis on the tomato types that are most vulnerable to poor fruit set.
- Relative performance of Bta and Btt/Btd in commercial tomato crops.

The work done during the first year of this two-year project included studies into Bta colony development, Bta flight activity / flower visitation and tomato flower / pollen development.

Summary

What was done

The original intention was to work with Bta colonies from one commercial tomato crop throughout the 2019 growing season and compare them to Bta colonies that had been placed outside in a more natural habitat. However, it soon became clear that there was large inherent variation in the numbers of bees present in the delivered hives and this confounded our experimental designs. We were forced to modify our original plans and subsequently sampled hives as opportunities arose to explore potentially informative situations. Overall, we covered more combinations of sites, tomato cultivars and bumblebee suppliers than originally intended but we had to compromise on factors such as the time the colony had been in position on site.

Forty eight bumblebee hives were sampled from three commercial tomato crops (cv Piccolo in the Midlands, cv Sweet Jane in West Sussex and cv Funtelle in East Yorkshire) and a natural habitat at Warwick Crop Centre (WCC). Upon receipt at the WCC laboratory, each hive was frozen, the colony destructively sampled and the numbers of bees, brood and eggs recorded. While our counts inevitably included some bees that were already dead inside the hive at the time of sampling, we believe that this method did provide a good indication of the size of each colony.

As the season progressed, it became clear that it would be useful to identify a best case scenario for Bta against which we could compare other situations. Our starting point for this scenario was a crop of baby plum tomatoes (cv Funtelle) in which there had been near perfect fruit set during the preceding five months. A preliminary investigation identified two Bta colonies which stood out as performing much better than any others in that crop. The study was done during a prolonged period of moderate weather when neither plants nor bumblebees had been subjected to stressful conditions. Bee flight, flower development and pollen flow were carefully monitored from sunrise until evening. We thus produced a ‘positive base-line’ against which we could compare flights from hives in other tomato crops, with less successful fruit set, and hives positioned in a natural habitat.

Bta hives were set up in an old redundant orchard in such a way as to simulate natural conditions with free access to a variety of flowering plants that grew in the vicinity. Bee traffic was first monitored from dawn to sunset recording all exit and entry flights. The same hives were observed on a further four occasions to determine the effect of weather on flight. Similar ‘snap shots’ of daily bumblebee flight activity were recorded in 31 hives over three occasions in a cherry tomato crop (cv Piccolo) in the Midlands, 8 hives in a cherry tomato crop (cv Sweet Jane) in West Sussex and 11 hives in a baby plum tomato crop (cv Funtelle) in East Yorkshire. In addition to these ‘snap shots’ of daily traffic, an additional study explored flight activity over the life of colonies both in the orchard and in the crop in the Midlands.

A preliminary study was organised to refine techniques for monitoring flower visitation ahead of tomato crop-scale trials in 2020. GoPro cameras were attached to roof support posts and aimed at developing flower trusses in the tomato crop. Flower development and bumblebee visitations were recorded from sunrise to sunset. The results were determined by replaying the recordings at increased speed in the laboratory.

Our experiments in 2018 indicated that the anthers of an individual tomato flower were generally able to produce many more pollen grains than were needed to fertilise all the ovules in the ovary of the same flower. However, it was possible that not all of the pollen grains were viable. During 2019, we concentrated on researching the methods available for assessing pollen viability ahead of more detailed experimentation in 2020.

Summary of Bta colony development, flight activity and flower visitation

There was considerable variation in the numbers of all Bta life stages in the colonies destructively examined regardless of the origin, age and / or history of those colonies. Prior to 2015, we had come to expect Btt/Btd colonies in tomato crops to show a peak in the number of adults at about six to eight weeks, after which the colony switched from rearing sterile workers to producing a small number of sexual males / females, and the total number of adult bees subsequently began to decline. No such pattern was seen in the data set from these tomato crops. In fact, the average numbers of adults and brood per hive remained relatively small and the development pattern over time was quite flat, which was broadly consistent with our observations from 12 tomato production sites in 2018. In contrast, hives collected from the outdoor habitat contained substantially more adult bees / brood than those from the glasshouses, and colony development did follow the pattern to which we had previously been accustomed.

Bta flights between late Spring and early Autumn generally began 2-3 hours after sunrise regardless of hive location and temperature. The frequency of exit flights increased during the morning, peaked at around 11:00 hrs, remained at that level until late afternoon and then declined into the early evening. Exit flights within one hour of sunset were rare but return flights continued until sunset. Bta flight activity in the outdoor habitat was substantially greater than in the glasshouse tomato crops.

The combined observations of colony development and flight activity reinforced the opinion that Bta simply do not like tomato plants! When we take these results along with the data obtained from estimates of bee colony size done the previous year, it is clear that bee colonies placed in tomato crops do not ‘take-off’ in terms of rearing brood and producing new foragers in the same way that a colony would normally do when held outside under more natural conditions. The colonies go into decline at about two weeks from being placed in the glasshouse. Therefore it is likely that most of the pollination is being done in tomato crops by adult forager bees that are present in the hive at the time of delivery, or which emerge shortly after delivery of the hive.

The GoPro camera method was successful in recording bumblebee flower visitation in a commercial crop. That technique, together with the Arnia remote hive monitoring system currently undergoing refinement, should become useful tools when we compare Bta and Btt/Btd flight activity and flower visitation in commercial crops in 2020.

Summary of tomato flower development and pollen production

Our ‘positive-base line’ study provided a detailed illustrated record of flower production and pollen flow in a crop of baby plum tomatoes (cv Funtelle). This record is available in the main project report and will be used by our team for reference in future trials. Significant visual changes were only observed to three flowers on the monitored truss. The first flower to open had clearly been visited by bees the previous day and produced no discernible quantity of pollen. The second flower to open became fully reflexed 3 hours after sunrise and began releasing pollen during the following 30 minutes. This continued until mid-afternoon when the flower began to close. The third flower became fully reflexed 4.5 hours after sunrise and started releasing pollen 30 minutes later. This continued into the evening. Pollen was thus available from two flowers per truss per day, in overlapping sequence, starting about 3 hours after sunrise and continuing until early evening. Bumblebee flights coincided with pollen availability. These observations were broadly comparable to those made in a crop of cv Piccolo in 2018. However, in cv Piccolo there were many more flowers per truss than cv Funtelle and up to four of them could be open at any one time. There appeared to be considerably less pollen within cv Piccolo flowers and it was more difficult to release by manual action.

Studies into tomato pollen production, availability and viability are continuing into 2020. It is interesting to note that several of the methods described in older published papers did not prompt germination of pollen from modern tomato cultivars in our experiments in 2019. We will continue to refine methods and then assess the viability of pollen from cv Piccolo under a range of temperature regimes during the coming growing season.

Financial Benefits

UK growers have become dependent upon the financial benefits from using biological pollination. It is difficult to generalise about the value of UK tomatoes, due to the wide range of products and production methods, but if we assume an average farm gate value of £850k / ha / season, then the total value of the British crop is about £162m / season. The loss of set due to inadequate pollination on just two trusses per plant (*i.e.* equating to 5% of annual production) is about £45k / ha / season or £8.6m across the whole British industry. The TGA's original investment appraisal for the first phase of this series of projects demonstrated a potential payback from just one hectare of crop in one growing season. If this project is successful in identifying and redressing the issues which lead to inadequate pollination equating to a 5% loss of annual production then, if extrapolated to the whole industry over a 5 year period, the potential cost:benefit of this series of three PE 031 projects is greater than 1:250.

Action Points

As an interim report, half way through a two-year project, this document is intended to provide an update on progress towards the ultimate objectives rather than provide firm recommendations on changes to be implemented by industry. Nonetheless, in the short term, the TGA can advise growers to:

- liaise with their bumblebee supplier to produce a hive input programme that compensates for the shorter colony life of Bta bumblebees.
- check Bta colonies regularly to ensure that they are still active.
- check pollen flow from flowers when conditions are deemed to be stressful for plant growth.
- 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

British tomato growers had successfully pollinated their crops with two non-native sub-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. NE 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.

As a consequence of the NE decision, growers had to switch to the British native sub-species, *B. terrestris audax* (Bta). The use of Bta in 2015/16 proved to be far from the reliable and maintenance-free experience to which the industry had become accustomed. For example, in 2015 one grower estimated poor fruit set cost his business over £50k / ha over a two month period. Several other growers suffered such poor results that they reverted to labour-intensive manual methods of pollination that had not been used since bumblebees were first used.

Prior to commercially reared bumblebees being introduced to British tomato growers in 1989, the three producers had 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 Btt from central Europe and Btd from south east Europe. 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. As a consequence, there is no attempt to distinguish between Btt and Btd in this report. 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.

At least one of those three producers, Brinkman Bunting Bumblebees BV, had tested and dismissed Bta during the screening process in the 1980s due to inferior performance (Griffiths, pers. com. 1990). British tomato growers are keen to use Bta if this can be done without significant economic loss and have instigated several studies in an attempt to improve the situation.

In 2015, the TGA / AHDB commissioned an independent review of the scientific literature relevant to the effects of releasing non-native sub-species of bumblebees as pollinators in commercial crops (AHDB project PE 026). The resulting peer-reviewed paper (Chandler *et al.* 2019) highlighted strengths, weaknesses and gaps in the current knowledge base. The authors 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 Bta to be complex with significant differences between populations from different parts of the British Isles (Moreira *et al.*, 2015). Results of mitochondrial COI sequence analysis and microsatellite analysis had found the COI haplotype normally associated with *B. terrestris* populations in mainland Europe to be present in populations from various parts of the British Isles. This included areas, such as the west of Ireland, where use of commercial bumblebees is very rare. This genetic information, along with apparent hybridisation among European mainland sub-species, raises questions about the validity of the current classification of *B. terrestris* sub-species. This in turn questions whether British *B. terrestris* populations should be assigned to any of the sub-species as currently defined. To shed more light on this issue, the project team have organised a spin-off study to investigate the molecular structure of some key populations of *B. terrestris* (Chandler & Prince, pers.com., 2019).

In 2017, the TGA Technical Committee 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. The full results may be found in AHDB project PE 031 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’, which made up 76.9% of UK production, were most commonly affected by poor set despite the use of extra Bta hives.
- About one third of growers reported an apparent lack of Bta activity during normal working hours. One grower believed that this was because Bta forage very early in the morning and their activity was poorly synchronised to peak pollen release / flow in tomato flowers.

In 2018, the TGA TC organised a short (6 months) AHDB-funded practical study to investigate some of the factors raised by the tomato growers in the survey. The full results may be found in AHDB project PE 031a report (Jacobson *et al.*, 2018a) and summarised in a subsequent article (Jacobson, 2019). In summary:

- Most Bta colonies went into decline soon after placement in tomato crops. This was in contrast to previous experience with Btt/Btd colonies. The only common factors across 13 production sites were Bta bumblebees and tomato plants; thus suggesting that the former either do not like the tomato growing environment or tomato plants do not provide them with a satisfactory food source.
- A study of Bta flight activity was done at three sites: a crop of cv Piccolo at Warwick Crop Centre, a commercial tomato crop and outside in an old redundant orchard. 'Bee traffic' was recorded by manually counting bees entering and exiting the hive from sunrise to sunset. Activity followed a similar pattern regardless of hive location or temperature, with the first activity just after sunrise, rising to a peak between 11:00h and 14:00h. Flights within one hour of sunset were rare. One notable feature was that activity in colonies in tomato crops was considerably lower than outdoors. This aspect of Bta behaviour requires further investigation.
- A system to remotely monitor Bta activity within hives was developed and tested in prototype form. Although the system required some further refinement, it was anticipated that it could allow researchers to record Bta within-hive and foraging activity in real time from their own laboratories.
- A preliminary study of flower development and pollen production was done in cv Piccolo. Each flower opened fully with its petals reflexed on two successive days releasing most pollen on the first day between 12:30h and 13:30h. This was reasonably well matched to Bta flights although some flowers peaked much earlier or later. The anthers of each flower had the potential to produce many more pollen grains than required to fertilise all the ovules in the ovary; *eg* 20,000 pollen grains but fewer than 120 seeds! However, it is possible that not all of these pollen grains were viable and capable of fertilising an ovule. Furthermore, pollen was more difficult to extricate manually than from flowers of larger fruiting cultivars and it is not yet known how efficiently this is done by Bta.

Based on the results from the above studies, the TGA TC organised the present AHDB-funded project (PE 031b) to further investigate the following:

- Activity of Bta in commercial tomato crops in the UK.
- Effects of high temperatures on within-hive behaviour of Bta and Btt/Btd.

- Effects of high temperatures on pollen production / viability with emphasis on the tomato types that are most vulnerable to poor fruit set.
- Relative performance of Bta and Btt/Btd in commercial tomato crops.

This work will be completed over two years ending in December 2020.

In 2019, the TGA TC repeated the 2017 survey to determine whether growers' perception of Bta performance had changed during the interim period. The full results of the survey, which included all the growers questioned in 2017, are beyond the scope of this report but the following extracts provide a useful update (Jacobson, pers. com., 2019).

Grower perception of bumblebee performance was based on a 1-5 scale where 1='good' and 5='financial loss despite all measures taken' (Figure 1). The overall mean score for three periods were: pre-2015 = 2.03, 2015 to 2017 = 3.16 and 2017-2019 = 2.96 (Figure 1). The slight improvement in perceived Bta performance in the 2017-2019 period may be attributed to improved Bta breeding stock and / or improved grower understanding of the in-crop management of Bta. The breakdown of mean scores into different tomato types in 2019 showed: cherry (3) > cocktail (2.9) > baby plum (2.8) > classic (2.2) (Figure 2).

Figure 1. Growers' overall perception of Bta performance between 2017 and 2019

Impression of bumblebee performance	Good	Good with occasional extra hives required	Some extra hives usually required	Extra hives frequently required & some manual pollination	Poor fruit set despite action in category 4 and some financial loss
Category / Score	1	2	3	4	5

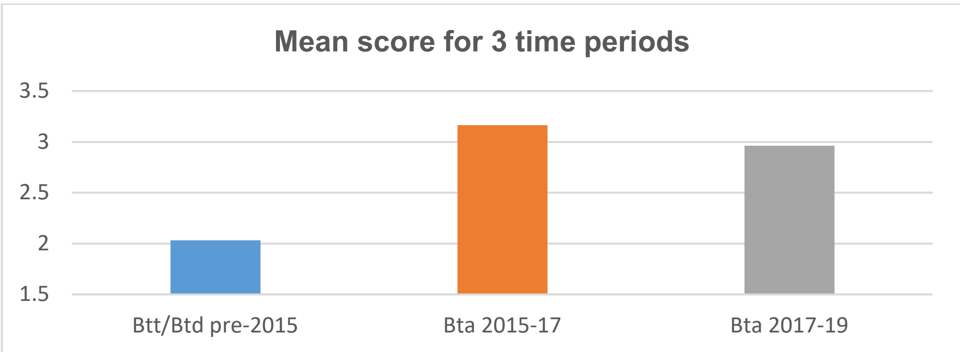
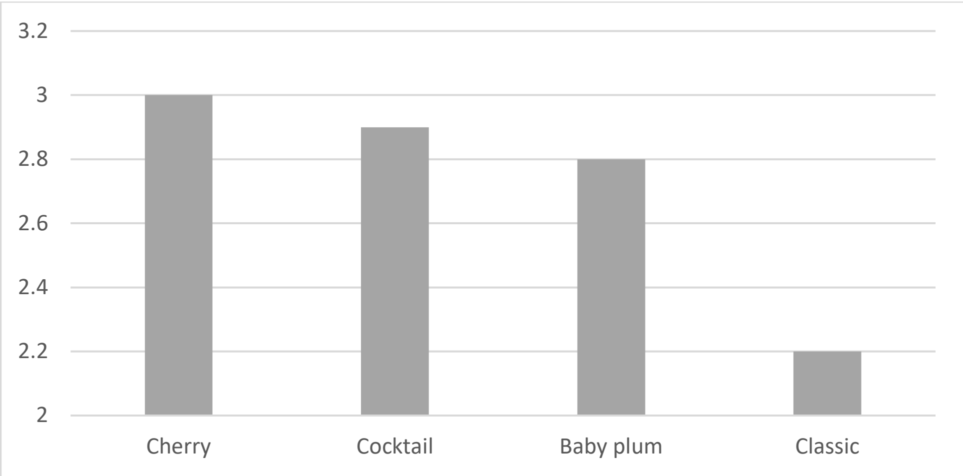


Figure 2. Mean of all grower scores for the main tomato types in 2019



The studies from AHDB project PE031b during 2019, which are included in this report, cover aspects of:

- Bta colony development
- Bta flight activity and flower visitation
- Tomato flower and pollen development

Materials and methods

Colony development: quantifying the numbers of bees within hives taken from glasshouse tomato crops and an outside situation

The original intention was to sample Bta colonies from one commercial tomato crop throughout the 2019 growing season and compare them to Bta colonies that had been placed outside in a more natural habitat. However, it soon became clear that there was large inherent variation in the numbers of bees present in the delivered hives and this confounded our experimental design. We modified our original plan and subsequently sampled as opportunities arose to explore different scenarios. Overall, we covered many more combinations of sites, tomato cultivars and bumblebee suppliers than originally intended (Table 1) but had to compromise on factors such as the time the colony had been positioned on site.

Table 1. Details of the origin of the hives sampled in the colony duration study.

Bee type	Site	Crop / location	Bee supplier	No. of hives
Bta	WCC	Old redundant orchard	1 (via agent)	2
Bta	Evesham	Cherry (Piccolo)	1	32
Bta	East Yorks	Baby plum (Funtelle)	1	6
Bta	West Sussex	Cherry (Sweet Jane)	2	8

Upon receipt at the WCC laboratory, each hive was frozen, the colony destructively sampled and the numbers of bees, brood and eggs recorded. This method provided an absolute count of the numbers of bees of different life stages within the hive but it was not possible to reliably distinguish between bees that were alive or dead in the colony at the time the hive was taken for the count. On the one hand, bumblebee workers have been shown to engage in hygienic behaviour in which corpses are removed from the colony or placed in middens (Munday & Brown, 2018). However, our personal experience of inspecting active colonies in situ indicates that dead bees are sometimes allowed to accumulate within the hive. This view is reinforced by observations of the very poor condition of some of the dead bees found in the hives after freezing. In preliminary studies, we have investigated alternative methods of counting bees by first removing live adult bees from colonies, either by chilling the hives to put the bees into torpor and then removing them under a red light, or by putting hives within mesh cages and removing the wadding so that flying bees were captured in the cage. However, those methods also had shortcomings and were found to be less accurate than freezing the colony before counting. While our counts inevitably include some bees that were already dead inside the hive at the time of sampling, we believe that they do provide a good indication of the size of that colony.

Bta flight activity and flower visitation

As with the ‘Colony Development’ study described above, our original plans were modified to take into account the initial findings and, in particular, the inherent variation in numbers of bumblebees present in delivered hives. As the season progressed, it became clear that it would be useful to identify a best case scenario for Bta against which we could compare other situations. Our starting point for this scenario was a crop of baby plum tomatoes (cv Funtelle) in which there had been near perfect fruit set during the preceding five months. A preliminary investigation identified two Bta colonies which stood out as performing much better than any others in that crop (*i.e.* more than 5x the flights than the overall mean at peak flight time). The study was done on a pleasant summer day during a prolonged period of moderate weather when neither plants nor bumblebees had been subjected to stressful conditions. We thus produced what may be described as a ‘positive base-line’ against which we could compare flights from hives in other tomato crops, with less successful fruit set, and hives positioned in an orchard.

Establishing a ‘positive base-line’ for Bta performance

Bumblebee flight activity from two standard Bta hives and tomato flower development were observed simultaneously in a commercial tomato crop (cv Funtelle) in East Yorkshire during August 2019. Bee traffic in and out of each hive was recorded from sunrise at 05:22 hrs until 18:30 hrs. The departure / arrival time of flights was recorded and it was noted whether returning bees were carrying pollen. A truss positioned above the hives was selected and marked at the beginning of the day. This was the first fully expanded truss and was typical of trusses in a similar position on all plants. It was examined and photographed (but not handled) at intervals of 30 minutes throughout the day and the presence of open flowers recorded. The presence of brown markings on anther cones were used to determine whether bumblebees had visited the flowers. Each time the truss was examined, equivalent open flowers on neighbouring trusses were tapped over a dark coloured card and pollen flow noted (Jacobson, 2007).

Recording bumblebee flight activity in other situations

Two standard Bta colonies were placed in the old redundant orchard on the farm at WCC in mid-May 2019 and replaced at four-week intervals. The hives were housed within a corrugated plastic box and insulated with hay bales and tarpaulin for weather protection and to replicate the underground nesting behaviour of Bta. These hives were set up to simulate the natural conditions in which Bta colonies live in the wild with free access to a variety of flowering plants that grow on the WCC farm. Adult bumblebee traffic was recorded manually or by GoPro video recordings, either from sunrise to sunset or from 11:00 hrs to 15:00 hrs (*i.e.* the time when peak activity had previously been identified), on several occasions. On the first occasion (20 May 2019), flight activity was observed between dawn (05:04 hrs) and sunset (21:04 hrs) and recorded as the times at which bees exited /entered the hives. The counts were then aggregated as numbers of departures / arrivals per 30 minutes. The same hives were observed on a further four occasions to determine the effect of weather on flight. In addition to these ‘snap shots’ of daily activity, colonies were observed one to four times per week over nine weeks (*i.e.* from 29 July to 17 September) to determine activity over the life-time of the colonies. Similar ‘snap shots’ of daily bumblebee flight activity were recorded in the following four commercial tomato crops:

- A total of 31 Bta hives in a cherry tomato crop (cv Piccolo) at Evesham on 28 March, 9 April and 3 June 2019. An additional study at this site explored activity over the life-time of Bta colonies by comparing mean numbers of flights from colonies that had been positioned in a tomato crop for 1, 2, 3, 4, 5, 6, 7 or 8 weeks.
- 8 Bta hives in a cherry tomato crop (cv Sweet Jane) in West Sussex on 18 June 2019.
- 11 Bta hives in a baby plum tomato crop (cv Funtelle) in East Yorks on 1 August 2019.

Flower visitation in a commercial crop

A preliminary study was organised to refine techniques ahead of crop scale trials in 2020. GoPro cameras were attached to roof support posts and aimed at developing flower trusses in a commercial tomato crop (cv Piccolo) near Evesham on 25 September 2019. Flower development and bumblebee visitations were recorded from sunrise to sunset. The results were determined by replaying the recordings at increased speed in the laboratory.

Further development of the Arnia remote monitoring system

Previous studies with ‘Arnia Hive Monitors’ in 2018 had strongly indicated that we could adapt their honeybee remote monitoring system (RMS) to work with our much smaller bumblebee colonies. The RMS not only had the potential to provide continual and more detailed information on Bta activity than labour intensive manual counts but could also provide information on hive environment / health; thus providing a valuable tool for future studies. In order to provide further data for calibration of the RMS, two monitors were set up in a commercial nursery - one with a standard Bta hive and the other with a ‘dummy’ hive. The latter consisted of an empty hive box containing additional cotton wool. Other calibration tests were set up in a controlled environment room at a standard temperature and humidity (20°C, 70%RH) and a known weight (700g). Unfortunately, these studies were thwarted by issues related to the transfer of data and external acoustic interference. All data has been sent to Arnia so that they can (i) adjust the settings on the load cells and environmental probes to ensure that units perform consistently and uniformly, (ii) recalibrate the load cells so that weight readings can be adjusted for fluctuations in temperature, and (iii) the gains on the microphone are modified to exclude external noise. No further information is available for this report but the studies will carry over into 2020.

Tomato flower and pollen development

Our experiments in 2018 indicated that the anthers of an individual tomato flower were generally able to produce many more pollen grains than were needed to fertilise all the ovules in the ovary of the same flower (Jacobson *et al*., 2018a). However, it was possible that not all of the pollen grains were viable. If true, this could be contributing to the problem of missed set in cv Piccolo. During 2019, we concentrated on researching the methods available for assessing pollen viability, of which the most useful are shown in Table 2. Other references that were consulted included Charles (1962), Maisonneuve and Den Nijs (1984), Yunlong Lu, LiqinWei and TaiWang (2015), and Jayaprakash, Sheeba, Vikas, Sivasamy and Sabesan (2018).

Table 2. The main sources of information that led us to the methods of assessing pollen viability that we shall trial in this project

A. Published reviews of methods for assessing pollen viability	
Picken, 1984	He surveyed all research on fruit set, pollen production and pollen viability and concluded that the quantity of *viable* pollen formed was the most important criterion. The most reliable method of assessing pollen viability was to place the pollen onto the stigmas of emasculated flowers and then to count the number of seeds in the resulting fruit. However, this method is slow and so *in vitro* methods including staining pollen and germinating pollen on artificial media have been used. These test viability but not the ability of pollen to germinate.
Stone *et al*., 1995	They reviewed tests to measure pollen viability and concluded that *in vivo* tests were more exacting than *in vitro* tests regardless of whether they used pollen tube growth within a style or seed set to quantify the response. As Picken also commented there would be a long delay before it was possible to determine whether the pollen was viable. Pollen germination, either in sucrose solutions or on agar media, overcomes the problem of delay. Some *in vitro* tests use the colour change of tetrazolium salts to indicate if there is any enzyme activity but a more reliable indicator is the fluorochromatic reaction test (FCR), in which fluorescein diacetate is hydrolysed to fluorescein. If the cell membrane is intact, the fluorescein accumulates in the cell and is very visible when viewed with a fluorescence microscope. A positive test confirms there is enzymatic activity and an intact cell membrane within the pollen grain but it still only measures the *potential* for germination.
Rathod *et al*., 2018	Compared five stain techniques for pollen viability and three *in vitro* pollen germination media on *Momordica*. A modified Alexander stain differentiated viable and non-viable pollen more accurately than any other stain. *In vitro* pollen germination and pollen tube growth was best in a medium containing sucrose, boric acid and calcium nitrite.

B. *In vitro* tests of pollen viability

B1. *In vitro* tests using a dye

Alexander,1969, 1980	He developed a chemical stain that was widely used to distinguish between aborted and non-aborted pollen.
Pressman *et al*., 2002	They used the Pressman *et al* (1998) technique on tomato pollen: viable grains stain purple while non-viable grains stain green. Flowers from tomatoes grown continuously at high temperature produced considerably less pollen and fewer grains were viable.
Peterson *et al*., 2010	They modified Alexander's staining procedure by removing chemicals that are now considered harmful. The resulting stain, based on Malachite Green, was simpler and quicker to use and continued to distinguish between aborted and non-aborted pollen grains.

B2. *In vitro* tests using fluorescence

Heslop-Harrison & Heslop-Harrison, 1970	They introduced the FCR test for testing pollen viability which measures the potential for pollen germination.
Abdul-Baki, 1992	Described a procedure for growing tomato pollen in a liquid growth medium to which 0.001% fluorescein diacetate (FDA) was added. Pollen viability could be evaluated within 30 minutes by determining the proportion of fluorescing pollen in a sample. He also measured pollen tube growth and found that germination and fluorescence were highly correlated. The procedure was simple and well adapted for routine screening but requires access to a fluorescence microscope.

C. *In vitro* tests for pollen germination

Murashige & Skoog, 1962	They developed a simple liquid medium in which cells could be cultivated successfully.
Brewbaker & Kwack, 1963	They modified the above liquid medium to study pollen germination and pollen tube growth and stressed the important role of calcium
Karapanos *et al*., 2006	They created an agar-based medium containing sucrose and PEG-6000; pollen grains were spread over the agar surface and those that developed pollen tubes were classed as germinated
Khanal, 2012	He used the Brewbaker & Kwak medium in petri dishes and released pollen from tomato anthers by vibrating flower pedicels with an electric toothbrush. Pollen grains with tubes at least as long as half the grain's diameter were recorded as having germinated.

We have accumulated the materials required to test those techniques and it is interesting to note that several methods described in older published papers have not prompted germination of pollen from modern tomato cultivars in our experiments. This work is continuing into 2020 when we shall select an appropriate method for assessing the viability of pollen from cv Piccolo when the plants are grown at high temperature as this is known to cause problems for pollen viability (eg Sato, Peet, and Thomas (2000), Sato, Peet and Thomas (2002), Sato, and Peet (2005), Pressman, Peet and Pharr (2002), Sato, Kamiyama, Iwata, Makita, Furukawa, and Ikeda, (2006)).

Results and Discussion

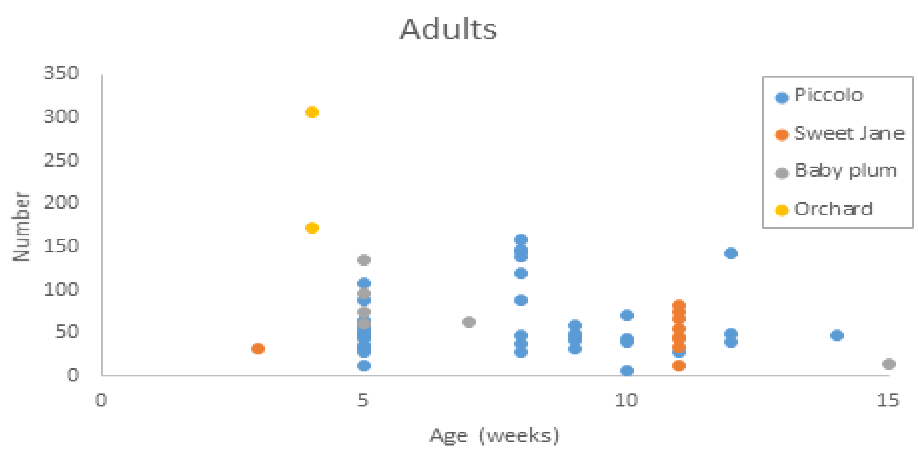
Colony development: quantifying the numbers of bees within hives taken from glasshouse tomato crops and an outside situation

There was considerable variation in the numbers of bees counted within the hives regardless of the location or age of the colony at the time of sampling. Numbers of adults, brood (*i.e.* larvae and pupae) and eggs recorded in hives from four different situations are shown in Figure 3. This included three different types of commercial tomato crops and one outdoor habitat. The data must be interpreted with caution as it was not possible to reliably determine whether the insects were still alive or already dead when the hive was frozen. However, the results do provide a good indication of the size of the colony and the presence / absence of eggs indicates whether the queen was still active and well nourished.

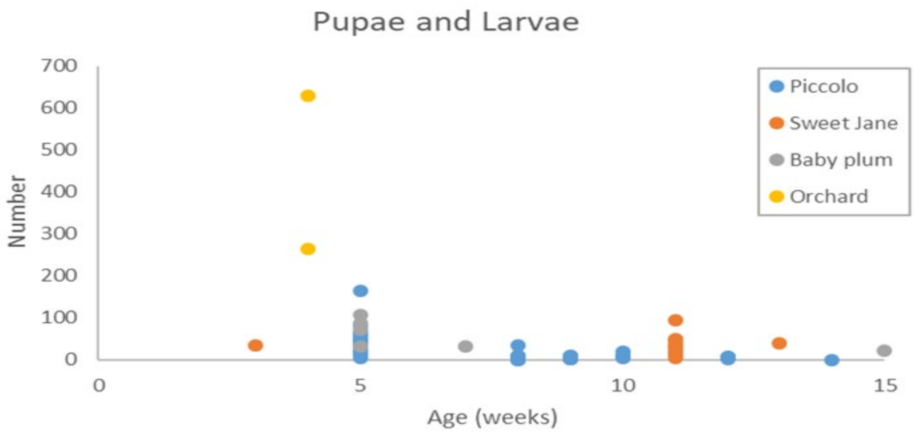
The numbers of adult bees ranged from 7 to 307, with the highest numbers being found in the colonies from the outdoor habitat. Numbers of brood ranged from zero to over 600, with highest numbers once again being found in the colonies from the outdoor habitat. Overall, 40% of the hives collected from the tomato crops contained less than 10 larvae / pupae and 6% contained none at all. Numbers of eggs ranged from zero to 61 (plus one anomalous count of 175). Overall, 79% contained less than 10 eggs and 73% contained none at all.

Figure 3. Number A) adult bees, B) brood and C) eggs recovered from colonies of various ages collected from three tomato crops and one outdoor habitat during 2019.

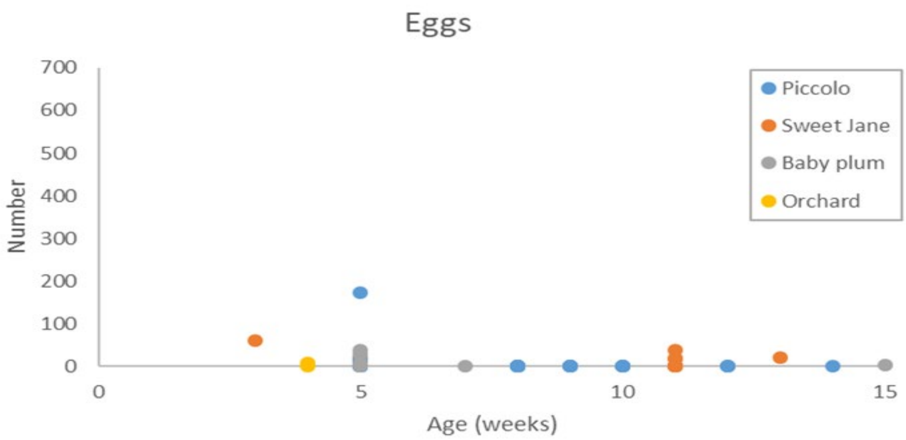
A)



B)



C)



Prior to 2015, we had come to expect colonies used for pollination in tomato crops to show a peak in the number of adults at about six to eight weeks, after which the colony switched from rearing sterile workers to producing a small number of sexual males / females and the total number of adult bees began to decline. Such a pattern was not seen in this data set. In fact, the average number of adult bees per hive, and the numbers of brood per hive, remained relatively flat when plotted against colony age.

For glasshouse bumblebees, there was no obvious link between colony size and tomato variety. The data from the colonies in the tomato crops was therefore combined and then compared with data from the outdoor habitat (Table 3) using only colonies within a narrow age range (3-5 weeks). Numbers of adult bees and brood from the outdoor habitat were greater than those from the tomato crops by factors of 4 and 8 respectively.

The large difference in brood production between the outdoor colonies and glasshouse colonies was particularly noteworthy, as was the observation that the one glasshouse colony sampled at three weeks also had very low brood production. This implies that – providing conditions are favourable - colonies held outdoors are capable of expanding quickly, with production of large amounts of brood at four weeks. The glasshouse colonies do not appear to have the same capability of producing large amounts of brood. In fact, the low numbers of adult bees and brood observed in the glasshouse bee hives was consistent with the findings from the 'tap-count' survey done in 2018 which estimated the relative size of adult Bta populations over about eight weeks (Jacobson, 2018). That survey showed that the colonies usually went into decline within about two weeks of being placed in the glasshouse, which would be consistent with producing few offspring.

Table 3. Mean number of adult bees, pupae, larvae and eggs recovered from colonies aged 3-5 weeks positioned within the outdoor habitat or commercial nurseries in 2019. Numbers in parenthesis represent the standard error of the mean.

	Adult bees	Pupae	Larvae	Eggs)	Total
Outdoor	240 (66.9)	284 (156.0)	163.5 (26.5)	3.5 (3.5)	691 (252.9)
Glasshouse	54.9 (6.97)	17.9 (2.93)	30.9 (4.39)	11.4 (3.91)*	115.3 (13.46)

* Mean omits the single anomalous figure

The size of the adult bumblebees found in the hives varied considerably. Examples are shown in Figure 4. The smallest bees are thought to be workers which usually remain in the hive tending the brood while the more robust bees forage for pollen. There was no consistent pattern relating the size of the bees to the size, age and / or activity of the colony but it was noticeable that the smallest individuals often predominated in the largest colonies.

Figure 4. Size range of adult Bta found in commercial hives.

Establishing a 'positive base-line' for Bta performance

A complete illustrated record of Bta flight activity linked to baby plum tomato (cv Funtelle) flower opening and pollen flow during daylight hours on 1 August 2019 is provided in Appendix 1. The results are provided in abridged form below.

Bta flight activity

The numbers of bumblebee flights in and out of two adjacent hives in the baby plum tomato crop during daylight hours on 1 August 2019 are shown in Figure 5a. The return flights are divided into 'with' and 'without' full pollen sacs. Recording began at sunrise (05:22 hrs). Thereafter, the day was broken down into 30 minute periods with each type of flight totalled within each period. A few bees made forays into the crop 2.5 hours after sunrise but flights out became more common during the following 30 minutes. Bees started returning with pollen 3 hours after sunrise. Exit flights peaked around 11:30 hrs (*i.e* 6 hours after sunrise) and continued at a comparable level until 17:30 hrs. Thereafter, exit flights declined into the early evening. Bees continued to return with pollen throughout the day although there was a dip in such flights mid-afternoon which could be consistent with a similar dip noted in flights in the orchard. Our calculations indicated that 12 bees had failed to return when observations ceased at 19:00 hrs (Figure 5b). They may have still been foraging or lost to the colony.

Figure 5a. Bta traffic from two hives during daylight hours in a baby plum tomato crop (cv Funtelle) on 1 August 2019. Flights are recorded as 'out', returning with pollen (IN-P) and returning without pollen (IN-X) within 30 minute periods throughout the day.

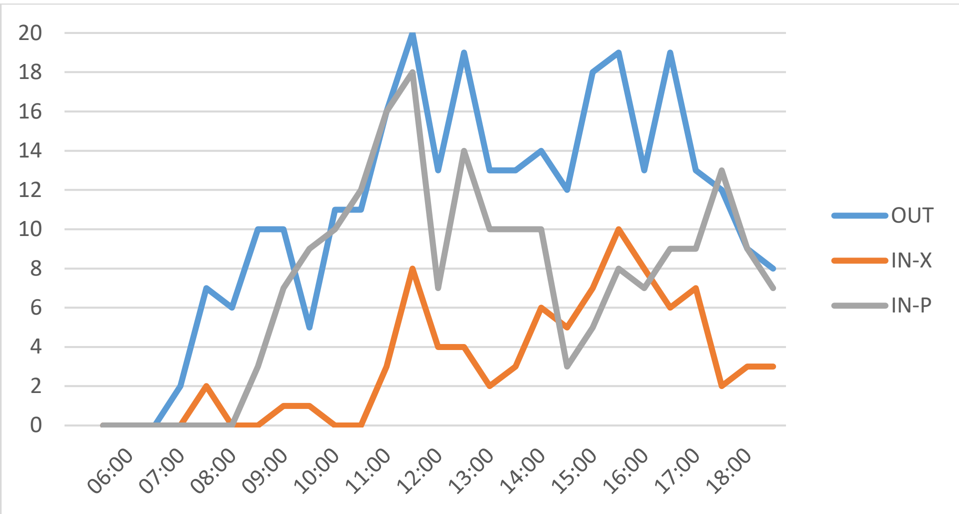
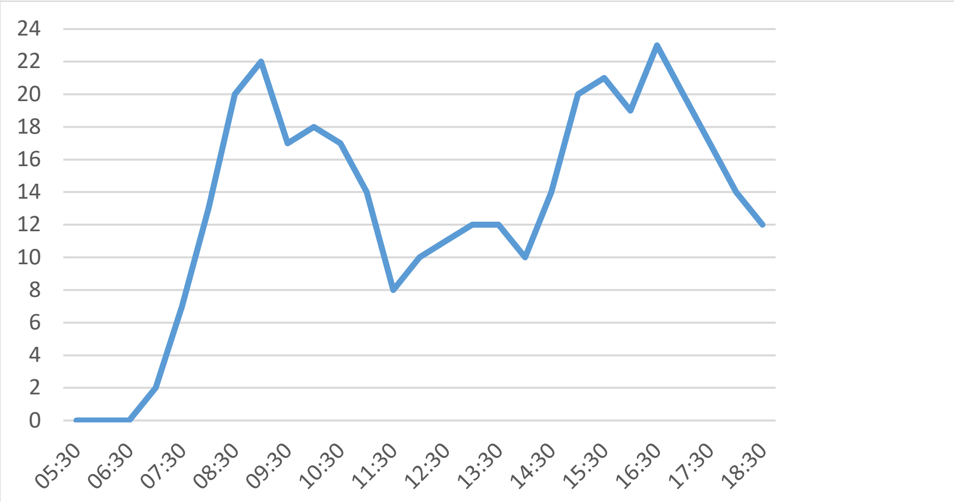


Figure 5b. Number of bees calculated to be out of the two hives within 30 minute periods throughout the day on 1 August 2019.



Flower opening and pollen flow

All flowers on the selected truss were closed at sunrise and remained so during the first hour of natural daylight (Figure 6). Significant visual changes were only observed to three of the flowers on that truss (labelled A, B and C in Figure 6), which was consistent with equivalent trusses on other plants in the same row.

- The oldest of those three flowers (designated A) began to open about 1.5 hours after sunrise. It had clearly been visited by bees the previous day. This flower was fully reflexed 3 hours after sunrise and remained so until mid-afternoon. It then began to close without having produced any discernible amount of pollen during the day (based on sampling equivalent flowers throughout the day).
- Flower B began to open 0.5 hours after flower A and was fully reflexed 3 hours after sunrise. There was no indication that it had been visited by bees the previous day. Pollen was not detected from flowers equivalent to B at that time but some bees were beginning to return to the hives with loaded sacs. Pollen was first detected from flower B 3.5 hours after sunrise and continued to flow until mid-afternoon when the flower began to close.
- Flower C began to open 3 hours after sunrise and was fully reflexed 4.5 hours after sunrise. Pollen flow began about 0.5 hours later and continued until early evening. Flower C is believed to have become the equivalent of flower A the following day.

In summary, pollen was produced by two flowers per truss, in overlapping sequence, starting about 3 hours after sunrise and continuing until early evening. Bumblebee flight activity coincided with pollen availability throughout the day.

Figure 6. The monitored truss as seen shortly after sunrise on 1 August 2019. The most significant visual changes during the day occurred to the three flowers labelled A, B and C.

Recording bumblebee flight activity in various situations

Bta flight activity in an outdoor habitat

Adult bumblebee traffic was recorded in and out of two hives positioned in an outdoor habitat on a pleasantly warm day in May 2019. Observations began at dawn (05:04 hrs), with sunrise at 05:49 hrs, and continued until sunset (21:04 hrs). Light levels ranged from 0 to 1025 w/m^2 (average 206 w/m^2) peaking between 11:00h and 14:00h and the temperature ranged from 7.6^{O}C to 25.8^{O}C (Average 14.5^{O}C) with peaks between 13:00h and 14:00h. The present data is restricted to the larger of the two colonies (Figure 7a). The first exit flight was about 1.5 hours after sunrise and lasted for 29 minutes. Peak flight activity occurred between 14:00h and 15:00h, which was after the sun was at its highest point (13:04 hrs). During that short period, 62 bees exited and 72 bees returned to the hive with full pollen sacs. Exit flights markedly declined during the last 2 hours of natural daylight and ceased by sunset. Entry flights continued until sunset, but not thereafter. The numbers of bees calculated to be out of the hive, presumably foraging, during each of those 30 minute periods, are shown in Figure 7b.

Figure 7a. Numbers of bees exiting and entering a hive in the outdoor habitat on 20 May 2019

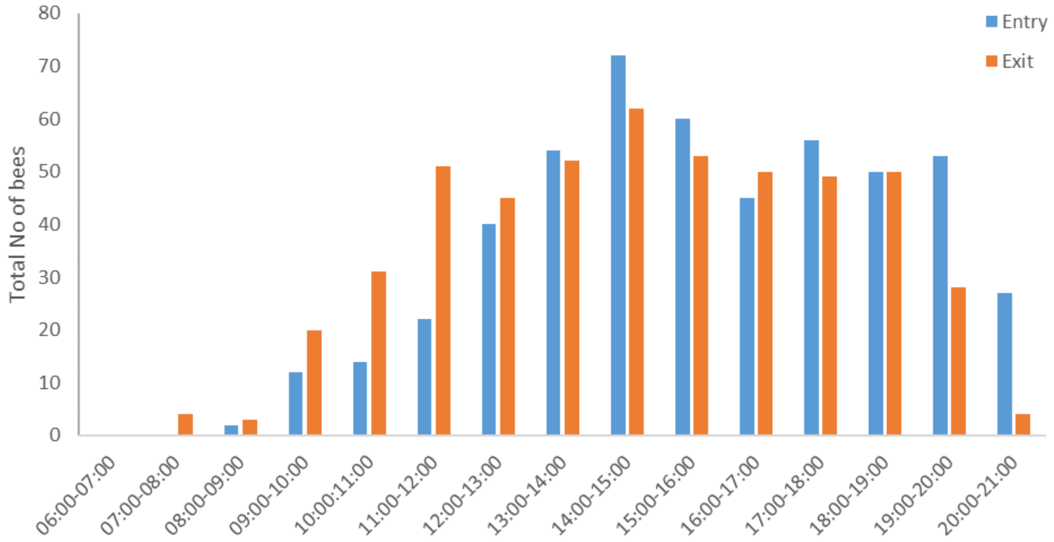
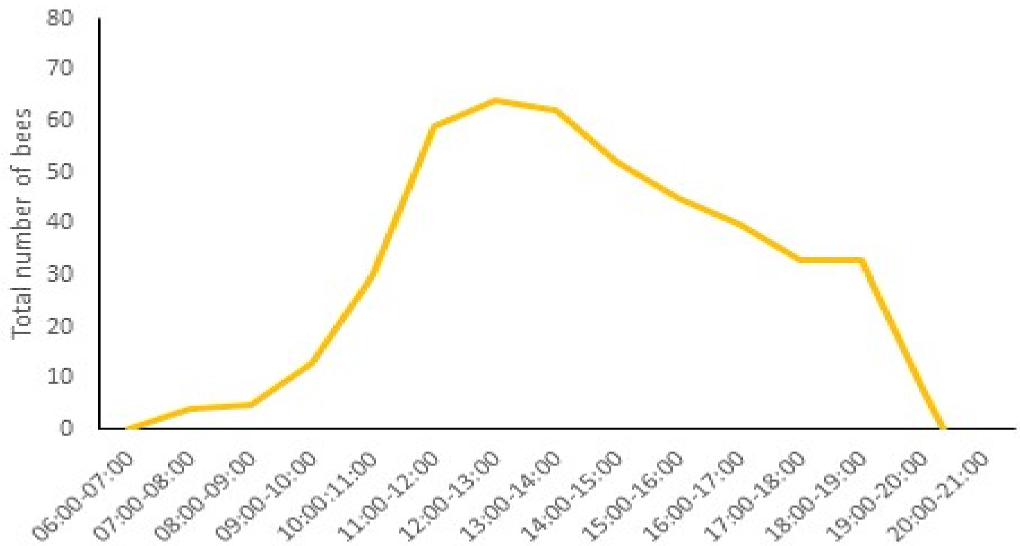
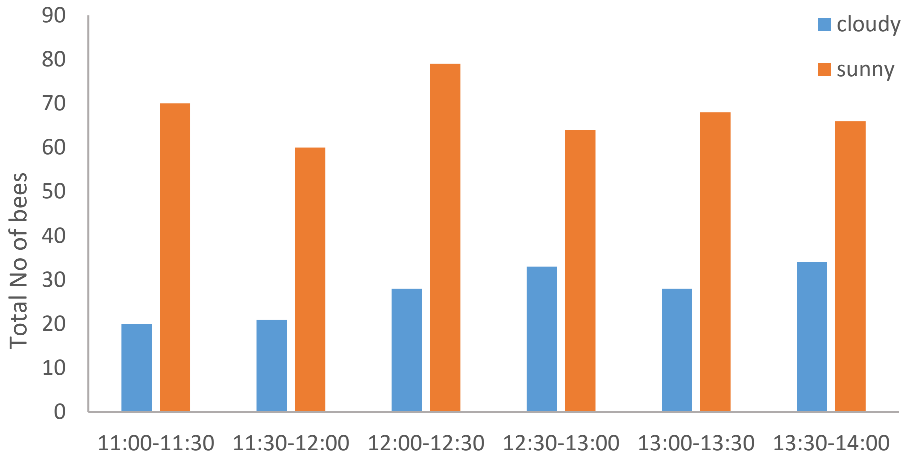


Figure 7b. Numbers of bees calculated to be out of the hive during each 30 minute period throughout 20 May 2019.



The same hives were monitored in the outdoor habitat on a further four occasions to determine the effect of weather. Observations were restricted to three hours in the middle of the day. The combined numbers of exit and entry flights for the larger colony, during six 30 minute periods, are shown in Figure 8. The bumblebees were considerably more active under sunny conditions.

Figure 8. Number of bees entering and exiting a hive in the outdoor habitat on cloudy and sunny days.



The Bta hives positioned in the outdoor habitat were monitored over nine weeks to determine the flight activity over the life of those colonies. The numbers of bees exiting and entering were recorded during 30 minute periods throughout the middle of the day. The results from the larger colony are presented in Figure 9. Flight activity peaked four weeks after placement and then steadily declined. There was still some activity at week 9. The ambient weather conditions throughout the 9 week period, which are summarised in Table 4, showed no obvious advantage to the bees at the time of peak activity.

Figure 9. Bta flight activity (exiting and entering) per 30 minute period in hives positioned in the outdoor habitat over nine weeks between 29 July and 17 September 2019 (error bars are where multiple readings were taken within a week).

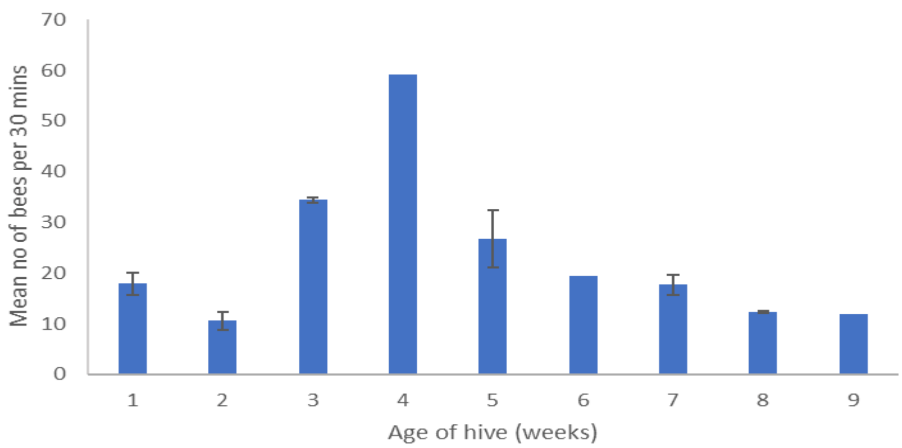


Table 4. Summary of weather conditions between 29 July and 17 September 2019.

Week	Max temp (°C)	Min temp (°C)	Rainfall (mm)	Sunshine (h)
1	23.62	12.07	11.70	5.93
2	24.43	14.05	0.00	7.65
3	24.35	17.31	4.00	5.35
4	21.67	10.00	0.53	7.07
5	28.90	14.00	2.20	5.10
6	No data			
7	20.38	10.98	0.80	7.02
8	24.21	13.58	0.00	4.03
9	20.62	6.45	0.07	8.74

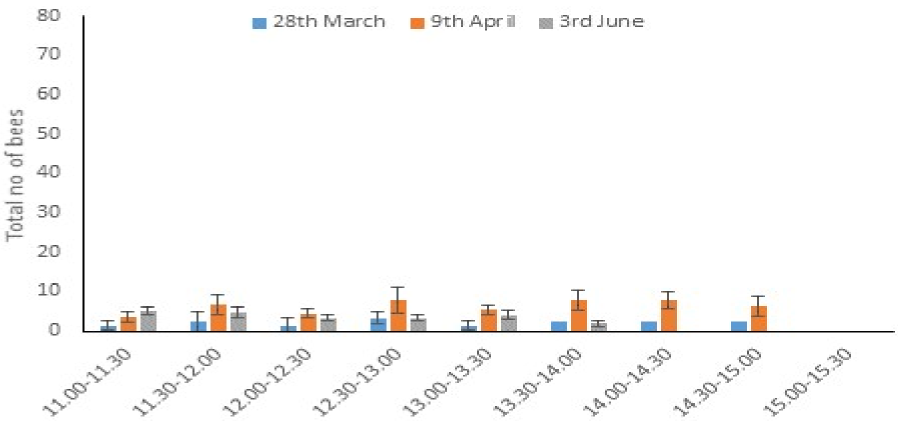
Bta flight activity in commercial tomato crops

As previously explained, formal experimental design was confounded by the large inherent variation in colony size encountered in hives on the commercial nurseries. This applied to all sites and suppliers regardless of time of year and age of hive. Much data has been collected which may be drawn on in later stages of this project. However, for the purpose of this annual report, we are focusing on results from three commercial tomato crops and presenting that data in a manner that may be compared to data collected from a more natural Bta habitat (Figure 10). Observations at one of the tomato production sites was replicated at three different times of the year while those in the natural habitat were done under two types of weather conditions. In each case, the combined numbers of exit and entry flights are shown for a sequence of 30 minute periods during the middle of the day. For reasons already given, we are interpreting these data with caution. However, it is clear that flight activity was broadly similar within all the tomato crops while they were all considerably less than in the bumblebees' natural habit even when weather conditions were poorer than ideal.

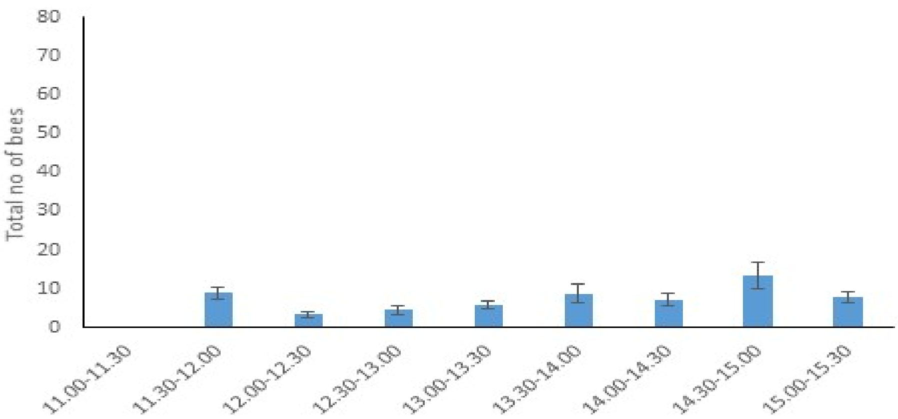
Bta flight activity data was compiled from numerous hives in the cv Piccolo crop during the course of the season and grouped according to the number of weeks since each hive was placed in the glasshouse. These data are presented here (Figure 11) in a form that is comparable to that already shown for activity in the bumblebees' outdoor habitat (see Figure 9). There was a small peak in flight activity two weeks after placement in the tomato crop. However, Bta flights in the outdoor habitat were far more numerous throughout the ages of the colonies.

Figure 10. Numbers of adult bees entering and exiting hives positioned in A) a cherry tomato crop (cv Piccolo), B) a cherry tomato crop (cv Sweet Jane), C) a baby plum tomato crop (cv Funtelle) and D) an outdoor habitat. The absence of bars at some time periods in charts A, B and C is because no data is available.

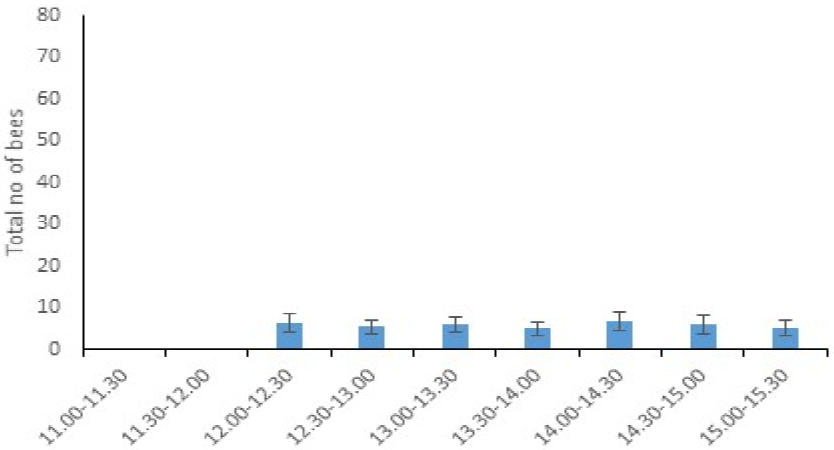
A) Mean of 31 hives



B) Mean of 18 hives



C) Mean of 11 hives



D) Mean of 2 hives on each occasion

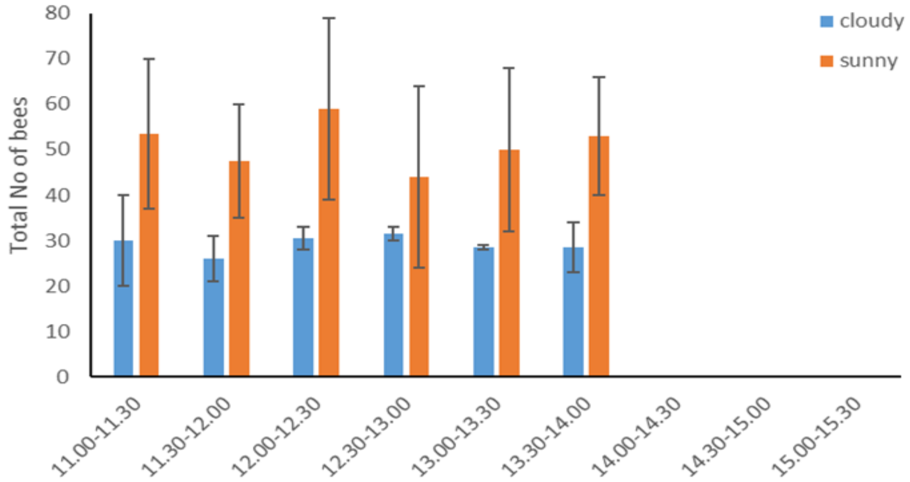
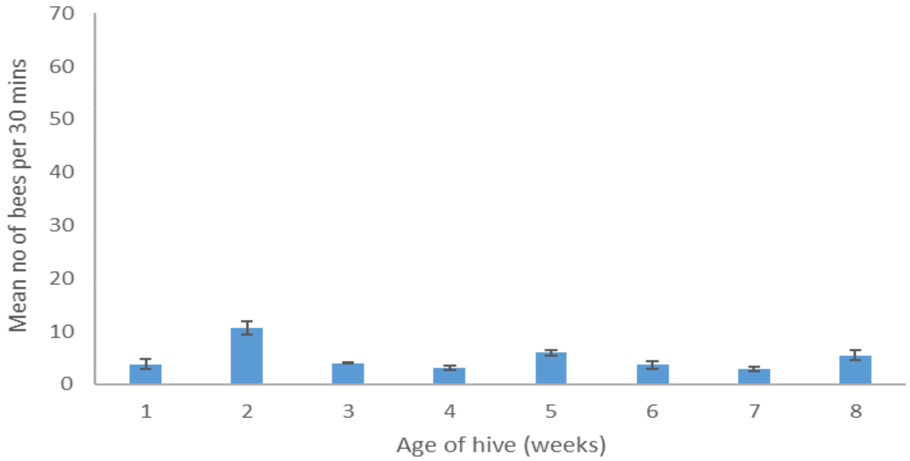


Figure 11. Bta flight activity (exiting and entering) per 30 minute period from hives of different ages a commercial tomato crop (cv Piccolo).



Flower visitation in a commercial crop

The GoPro camera technique proved to be successful and will provide a useful technique in studies in commercial crops in 2020. Fully expanded trusses, typical of trusses in a similar position on all plants, were recorded from 10.30 hrs to sunset. Bee visitations are shown in Table 5. No bee visitation was recorded on 10 of the 16 (*i.e.* 62%) observed trusses. Of a total number of bee visitations recorded, the first visitation to any truss was recorded at 13.02 hrs and the last truss visited was at 14.57 hrs. With the exception of one visitation, each bee flew directly to the open flower and spent 2-4 seconds on that flower before flying off. Only on one visitation did a bee visit two flowers consecutively on the same truss.

Table 5. Number of flower visitations by bees in a commercial nursery on 25 September 2019 as recorded by GoPro camera

Truss	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Flower visitation	No	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No	No
No visited					1	1	1	1			2	1				
Time of visit					13:02	13:07	13:50	16:47			14:48	14:57				
Duration (s)					3	3	2	4			2	2				

Conclusions

- The 2017 survey of tomato growers was repeated in 2019. It identified a marginal improvement in growers' perception of the performance of Bta, which could perhaps be attributed to improved Bta breeding stock and / or improved grower understanding of in-crop Bta management. Nonetheless, most growers still considered Bta to be less vigorous than the non-native bumblebees and more likely to fail to provide adequate pollination should any influencing factor be sub-optimal.
- There was large inherent variation in the numbers of adult bumblebees present in delivered hives and this confounded many of our experimental designs. It was often necessary to resort to investigating potentially informative scenarios as opportunities arose.
- There was considerable variation in the numbers of all Bta life stages in the colonies destructively examined regardless of the origin, age and / or history of those colonies.
- No obvious link was found between Bta colony size and the tomato varieties investigated. Furthermore, there was no indication of Bta colonies following the development pattern that we had come to expect with Btt/Btd. (see page 17). All hives collected from the outdoor habitat contained substantially more adult bees and brood than those from glasshouses. In addition, colony development in those hives followed the pattern to which we had previously become accustomed.
- Bta flights between late Spring and early Autumn generally began 2-3 hours after sunrise regardless of hive location and temperature. The frequency of exit flights increased during the morning, peaked at around 11:00 hrs, remained at that level until late afternoon and

then declined into the early evening. Exit flights within one hour of sunset were rare but return flights continued until sunset.

- Bta flight activity in the outdoor habitat was substantially greater than in glasshouse tomato crops.
- The combined observations of colony development and flight activity reinforced the opinion that Bta simply do not like tomato plants. When we take these results along with the data obtained from the 'tap-count' estimates of bee colony size done the previous year (PE 031a), it is clear that bee colonies placed in tomato crops do not 'take-off' in terms of rearing brood and producing new foragers in the same way that a colony would normally do when held outside under more natural conditions. The colonies go into decline at about two weeks from being placed in the glasshouse. Therefore it is likely that most of the pollination is being done by adult forager bees that are present in the hive at the time of delivery, or which emerge shortly after delivery of the hive.
- The 'Arnia Hive Monitors' honeybee remote monitoring system requires further refinement before it can be used with our much smaller bumblebee colonies but it still has potential to be a valuable tool in future studies in commercial tomato production sites.
- In a crop of baby plum tomatoes (cv Funtelle), pollen was produced by two flowers per truss per day, in overlapping sequence, starting about 3 hours after sunrise and continuing until early evening. Bumblebee flight coincided with pollen availability.
- A technique has been developed to record flower visitation by bumblebees in a commercial crop using GoPro cameras.
- Considerable resource has been devoted to perfecting a reliable method of assessing tomato pollen viability. Several methods previously reported in the scientific literature failed to prompt germination of pollen from modern tomato cultivars in our experiments. This work continues ahead of larger scale trials during 2020.

Knowledge and Technology Transfer

- Jacobson (2019). Not so Busy Bees. Article for ‘The Grower’ (Journal of AHDB Horticulture). Issue 244. Feb/Mar 2019.
- Chandler & Jacobson (2019). Presentation at the AHDB Protected Edibles Crop Day, Brownsover Hall, Rugby, 27 February 2019
- Chandler (2020). Presentation requested by AHDB at the Protected Edible Crop Day, 11 March 2020
- Jacobson (2019). Reports to TGA Technical Committee meetings:
    - Stoneleigh, 20 February 2019
    - Wellesbourne, 10 July 2019
- Jacobson, Chandler & Cockshull (2019). Annual Project Review. Presentation to AHDB and TGA TC, Stoneleigh, 4 December 2019

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APPENDIX 1

BOMBUS TERRESTRIS AUDAX FLIGHT ACTIVITY IN RELATION TO FLOWER OPENING AND POLLEN FLOW IN BABY PLUM TOMATO

The following illustrated notes record Bta flight activity linked to tomato (cv Funtelle) flower and pollen development throughout daylight hours on 1 August 2019.

TIME PERIOD	BEE TRAFFIC			FLOWER OPENING / POLLEN FLOW
	OUT	IN - no pollen	IN with pollen	
0500-0530	0	0	0	• Sunrise at 0522 • Bright sun through side wall of glasshouse • All flowers closed
0530-0600	0	0	0	• No obvious change
0600-0630	0	0	0	 Image 1 • All flowers still closed • Subsequent activity involved only the 3 labelled flowers
0630-0700	2	0	0	 Image 2 • Flower A just beginning to open. It had clearly been visited the previous day. • No other obvious changes

0700-0730	7	2	0	B  Image 3 • Flowers A&B both open to stage 3 • B had not been visited the previous day • No detectable pollen flow from equivalent flowers • In fact, there was no pollen flow from flowers equivalent to A all day and this will not be mentioned again
07:30-0800	6	0	0	• Little change
08:00-08:30	10	0	3	• Flowers A & B further reflexed (stage 4) • Flower C just beginning to open • First bees return with pollen but none detected by my method
08:30-09:00	10	1	7	C  Image 6 • Pollen flowing from flowers equivalent to B • Very faint visiting mark on Flower B • Flower C about 80% reflexed
09:00-09:30	5	1	9	• Flowers A, B & C further open but no other obvious difference
09:30-10:00	11	0	10	• Flower C at stage 4 but no pollen detected.

10:00- 10:30	11	0	12	C  Image 9 • A little pollen detected from flowers equivalent to C • There was a very faint visiting mark on Flower C (probably just happened). • Note the two flowers beyond C are at stage 2 and remained like this all day
10:30- 11:00	16	3	16	• Stronger visiting marks on Flower B • Otherwise little change • Conditions now overcast
11:00- 11:30	20	8	18	• Slightly stronger visiting marks on Flower C • Otherwise little change
11:30- 12:00	13	4	7	• No significant changes.
12:00- 12:30	19	4	14	• Conditions brighter again • Stronger pollen flow from Flower C equivalents
12:30- 13:00	13	2	10	 Image 14 • Good pollen flow from Flower B & C equivalents.
13:00- 13:30	13	3	10	• No significant changes.
13:30- 14:00	14	6	10	• No significant changes.
14:00- 14:30	12	5	3	• Flower B starting to close • Otherwise no significant changes

14:30-15:00	18	7	5	• Flower B slowly closing • Otherwise no significant changes
15:00-15:30	19	10	8	• Flower A well into stage 5. • Flower B just starting to close (Image 22) 
15:30-16:00	13	8	7	• Flowers A & B closing more rapidly • No obvious change with Flower C
16:00-16:30	19	6	9	• Flowers A&B closed. • No obvious change with Flower C  .
16:30-17:00	13	7	9	• Little change • Still some pollen flow detected from Flower C equivalents
17:00-17:30	12	2	13	• Little change in flower appearance • However, becoming very difficult to detect pollen • Nonethelees, bees are still returning with pollen
17:30-18:00	9	3	9	• No obvious changes
18:00-18:30	8	3	7	 Image 26 • Little change in flower appearance • I couldn't detect pollen by my method but some bees were still returning with full pollen sacs. • The image shows the state of the truss as we approached the end of the day.

