Project title:	Tomato: An investigation into poor pollination performance by the native bumblebee, *Bombus terrestris audax*
Project number:	PE 031
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Report:	Final report, December 2017
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Location of project:	RJC Ltd, Bramham, Wetherby, West Yorkshire Warwick University Various UK tomato production nurseries
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Date project commenced:	1 May 2017
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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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CONTENTS

GROWER SUMMARY

Headline

- This report summarises the experiences and opinions of growers representing 98% of the UK tomato production area.
- The results provide a foundation of knowledge upon which to build a practical research programme aimed at improving pollination with *Bombus terrestris audax* in tomato cultivars currently grown in the UK.

Background

Bumblebees were first introduced to British tomato growers in 1989 via trials in glasshouse crops on the Isle of Wight. 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) and *B. terrestris dalmatinus* (Btd). The British native sub-species, *B. terrestris audax* (Bta) was dismissed at that stage due to inferior performance. In the 27 years since the first release of non-native bumblebees in UK tomato crops, there has been no evidence of their establishment outside glasshouses or any detrimental effect on natural bumblebee populations.

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 not averse to using Bta if this can be done without significant economic loss. In the short term, the Tomato Growers Association (TGA) Technical Committee requested that an appropriate team be formed to conduct an in-depth survey of UK tomato growers to gather more precise information about the current situation. The results of that survey form the basis of this report. It is important to stress that the information presented here represents the views of UK tomato growers rather than the views of the authors of the report. In the longer term, the information generated from this survey will be used to provide a foundation of knowledge upon which a practical research programme can be constructed.

Summary

What was done

The study was done in three distinct stages with interim reports prepared after each stage. These reports were used by the project team to take stock of progress up to that point and determine how the project should proceed.

The first stage began by searching scientific and horticultural literature as well as interviewing consultants and scientists who had been involved with practical work on flowering and fruit set in tomato. In addition, tomato consultants who currently specialise in tomato ‘crop registration’ were interviewed with particular emphasis on ‘vegetative’ versus ‘generative’ plant growth and how plant condition might influence flower quality and fruit set. There followed visits to three UK tomato growers who had suffered serious difficulties with fruit set during 2015/16 to investigate the circumstances surrounding those problems. Finally, representatives of each of the three bumblebee supply companies were interviewed to obtain their opinions on how biological pollination had changed during the transition from non-native to Bta bumblebees. The combined findings were used by the team to produce a series of notes to help steer the subsequent grower interviews.

In the second stage, growers from a further ten tomato production sites were interviewed following the agreed procedures. All interviews were conducted by the same team member to aid consistency. Care was taken to ensure that the interviewer did not ask leading questions or inadvertently direct the discussion in a particular direction. However, the growers

were encouraged to develop any subject which they felt was particularly important in their situation. The second interim report was used by the team to refine the overall approach for the remaining interviews. Where necessary, further contact was made with the stage 1 and 2 growers to clarify any points that had not been adequately covered.

In the third stage, the same team member contacted all the remaining UK tomato growers and conducted interviews following the latest agreed procedures. In total, growers from 32 tomato nurseries were interviewed. This represented 186.2 hectares of crops which the TGA believe to be 98% of the UK glasshouse area currently devoted to tomato production. In fact, only one company failed to contribute to the survey. The sites ranged in size from 0.2 ha to 23.5 ha with an average of 5.8 ha. A third interim report was produced which was used by the team to tease out the key factors for inclusion in this AHDB final report.

Summary of findings

The term 'fruit set' is used in this report to denote flowers which reach anthesis (flower opening) normally and subsequently produce fruit of marketable size for that tomato type / cultivar. Very little research has been published on flowering and fruit set in tomato since the introduction of commercial bumblebees for pollination in the late 1980s. This reflects the reliability of the biological system between 1990 and 2015.

There are reported to be nine sub-species of *B. terrestris* in Europe and North Africa. Btt occurs naturally in continental Europe (north of the 45^{th} parallel) and is considered to be the 'type species' to which the others are compared. Btd is naturally found in south east Europe (from S.E. France to Iran) but has an overlapping range with Btt and some hybridisation has been reported in the common territories. Bta evolved within the British Isles. There is very little information in the scientific literature which quantifies differences in vigour between Bta, Btt and Btd. However, in one field experiment in southern England, colonies of Bta (reared from nest searching queens caught in the wild) and Btd (obtained from a commercial supplier) were found to have different nectar foraging performances, with Btd performing significantly better than Bta in four out of five study locations. This was attributed to the larger body mass of Btd.

The clearest overall message from British tomato growers in this survey was the general belief that Bta are less vigorous than the previously used non-native sub-species and more likely to fail to provide adequate pollination should any aspect of flower development or ambient conditions be sub-optimal for their performance. In fact, 72% of growers said they would prefer to return to using the non-native bumblebees.

No growers considered the performance of Bta to have surpassed Btt/Btd but 34% believed that their performance was similar - albeit with many more Bta hives being used than had previously been required with Btt/Btd. 28% of growers said Bta were poorer, 28% said much poorer and 9% said very much poorer than Btt/Btd. One grower estimated that poor fruit set had cost his business £50k / ha in 2015. Due to reduced confidence in bumblebees, 75% of growers now devoted more labour to monitoring fruit set than when they were using non-native bumblebees which was an additional cost to the business.

Planned Bta hive input schedules varied greatly between suppliers, sites, types / cultivars of tomatoes and length of growing season. A large proportion of the growers' interviewed said they had also required additional hives to those included in the planned schedule. In most cases (78%), the supplier accepted the growers' judgement and provided the extra hives without further investigation. In summary, 28% of growers occasionally ordered extra Bta hives while 69% said this was a usual or frequent requirement.

47% of growers thought that Bta colony life was shorter than they had come to expect from non-native bumblebees with estimates ranging from 2 to 6 weeks less than Btt/Btd. Shorter than anticipated colony life could result in gaps in the planned schedule with an associated breakdown in the continuity of bumblebee activity. This might help to explain why so many additional Bta hives are ordered during the season. Four growers reported improved results when they changed from fortnightly to weekly hive deliveries which was thought to reduce peaks and troughs in bumblebee activity.

44% of growers linked poor performance of Bta to poor foraging during hot environmental conditions. Such conditions were poorly defined but probably involved temperatures exceeding 28^{O}C around the heads of the plants for at least 5-6 hours. This may be a direct effect of temperature on the bumblebees or an indirect effect via flower development and quality of pollen. Most of these growers based their comments on subjective observations rather than actual measurements. The remaining 56% of growers did not express an opinion on the subject.

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 is underestimated. If correct, then Bta activity may not be very well synchronised to our understanding of pollen release / flow in tomato flowers. As yet, there is little evidence to support this theory but it must be investigated in more detail.

There is a common perception among UK growers that it is the greater size of Btt and Btd which makes them more effective pollinators. In reality, the physical features of the *B. terrestris* sub-species are quite varied and there is certainly some overlap between the sub-species that have been available to British growers. Furthermore, the non-native bumblebees that were being released immediately prior to 2014 were probably very different to those originally collected from the wild due to further selection, manipulation and possible hybridisation while in culture. It is entirely possible that Bta breeding stock may also become stronger as the producers select Bta queens which produce larger and more vigorous colonies.

All types of tomato can be affected by poor set but problems are most serious in smaller fruiting cultivars which produce more flowers. The plant may compensate for missed set by producing larger remaining fruit. Assuming the larger fruit are picked loose, sold by weight and still within the permitted size category, then some of the lost yield will be recovered. The issues are always more serious where tomatoes of any type are ripened on the plant and harvested as whole trusses. Apart from the obvious loss of fruit, missed set within a truss results in additional work at harvest and / or in the pack house in order to make the necessary adjustments to the trusses.

One grower, who reported a marked reduction in bumblebee efficacy since the switch to Bta, also reported the move at his site to small fruiting cultivars during the same period. This prompted the project team to look at the overall UK change to small fruiting varieties during the period that NE have 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%. This is clearly an important factor when considering the efficacy of bumblebees.

Six growers presented examples of exceptional cases where they believed the condition of the plants had been the underlying cause of poor set. Three were due to excessive vegetative growth resulting from i) a prolonged period without heat, ii) inappropriate growing conditions for a new specialist cultivar and iii) root mat disorder. Two cases were due to weak plants infected with pepino mosaic virus and the other due to excessive manganese in the water supply.

Apart from these exceptional cases, only 12% of growers considered that the condition of the plants could have been the underlying cause of poor set experienced during the last three years. Nonetheless, there is a strong belief among some consultants and growers that vegetative, rather than generative, tomato plant growth results in weaker flowers and poorer set. Although one grower believed that Bta are 'weaker' than Btt/Btd, he also thought that current crop husbandry practice led to poor pollen quality in modern tomato cultivars and this was the underlying cause of poor fruit set. In his opinion, if plants are strong and generative, then pollen flows freely and may not even need disturbance by bumblebees to pollinate. There can be little doubt that any future studies into pollination by bumblebees must also take into account the impact of environmental conditions on the condition of plants with particular emphasis on flower development and pollen quality.

One grower said pollen did not flow freely in humid conditions and speculated that Btt/Btd could cope with this but Bta could not. This should be further investigated.

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

Future studies into fruit set should focus upon:

- Bta biology and behaviour under different environmental conditions with particular emphasis on synchrony between bumblebee foraging and optimum pollen flow.
- Bta colony life in greenhouses and the impact this has on hive input schedules and frequency of hive deliveries.
- The influence of environmental conditions on flower quality and pollen flow in the small fruiting cultivars which now make up 76.9% of UK production.
- Changes in bumblebee usage and agronomic practice that will be required to optimise all aspects of pollination and fruit set.

SCIENCE SECTION

Introduction

Bumblebees were first introduced to British tomato growers in 1989 via trials in glasshouse crops on the Isle of Wight. 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 (*i.e.* to the UK) sub-species; *B. terrestris terrestris* (Btt) and *B. terrestris dalmatinus* (Btd). The British native sub-species, *B. terrestris audax* (Bta) was dismissed at that stage due to inferior performance. The results of that 'internal' company research were commercially sensitive and weren't published. In the 27 years since the first release of non-native bumblebees in UK tomato crops, there has been no evidence of their establishment outside glasshouses or any detrimental effect on natural bumblebee populations.

In 2014, Natural England (NE) produced a document which suggested that non-native bumblebees could escape from glasshouses and become invasive. They suggested that non-native sub-species could possibly hybridise with wild Bta leading to the introgression of genes from non-native sub-species into the Bta genepool and 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 Bt to wild bumblebees in the UK. This document formed the basis of an open consultation on a NE proposal to change the licensing regime relating to the release of non-native bumblebees in England. The TGA had serious concerns about the quality of the evidence base available to the policy review and this was highlighted in a formal response to NE. Nonetheless, following the 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. A preliminary survey of TGA members (by e-mail) at the end of that season indicated that 80% of respondents were ‘less than happy’ with Bta and rated their performance as only 60% of the non-native bumblebees. Some of the 20% of growers who reported adequate pollination by Bta said this had been achieved by using many more hives than had been required with Btt or Btd.

For the 2016 season, NE allowed two growers to use Btt or Btd for part of the season under a special license on condition that research was done to compare the performance of the various sub-species. Research protocols were agreed with NE which utilised bee counters to monitor activity at hive entrances. The results were inconclusive. However, the work did indicate that the life of the bumblebee colonies was shorter than growers had come to expect during the previous 25 years of reliable biological pollination. It is not known if this short life expectancy was experienced at other production sites.

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; *i.e.* reduced labour costs, improved fruit set, increased fruit size and better fruit shape, as well as complete truss formation in cultivars which are harvested and marketed as whole ripe trusses.

It is difficult to generalise about production levels and the financial value of British tomato crops due to the wide range of products supplied to our 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. Inadequate set on additional trusses would increase losses proportionally. This would not be sustainable and could force growers out of business.

British tomato growers are not averse to using Bta if this can be done without significant economic loss. In the short term, the TGA Technical Committee requested that an appropriate team be formed to conduct a more in-depth survey of UK tomato growers to gather more precise information about the current situation. In the longer term, this information will be used to provide a foundation of knowledge upon which a practical research programme can be constructed.

Materials and methods

The study was done in three distinct stages with interim reports prepared after each stage. These reports were used by the team to take stock of progress up to that point and determine how the project should proceed.

The first stage began by gathering background information about the process of flowering and fruit set in tomato. This involved searching scientific and horticultural literature as well as interviewing consultants and scientists who had been involved with practical work on these subjects in the 1970s and 1980s. In addition, tomato consultants who currently specialise in tomato 'crop registration' in several countries were interviewed with particular emphasis on 'vegetative' versus 'generative' plant growth and how plant condition might influence flower quality and fruit set. There followed visits to three UK tomato growers who had suffered difficulties with fruit set during 2015/16 and were known to have conducted internal studies into the performance of bumblebees in their own crops. At each of these sites, there were in-depth discussions to investigate the circumstances surrounding the poor fruit set. Finally, representatives of each of the three companies who had been supplying bumblebees in the UK for the previous 25 years were interviewed to obtain their opinions on how biological pollination had changed during that period. The combined findings were used by the team to produce a series of notes to help steer subsequent grower interviews.

In the second stage, growers from a further ten tomato production sites were interviewed following the procedures agreed by the project team at the end of first stage of the project. All interviews were conducted by the same team member to aid consistency. Care was taken to ensure that the interviewer did not ask leading questions or inadvertently direct the discussion in a particular direction. To that end, subjects were organised in the same order in each interview and similar emphasis was placed on every point. However, the growers were encouraged to develop any subject which they felt was particularly important in their situation. At the end of this stage, a second interim report was produced for discussion by the team and the overall approach was refined for the remaining interviews. Where necessary, further contact was made with the ten stage 2 growers to clarify any points that were not adequately covered in the first interview.

In the third stage, the same team member contacted all the remaining UK tomato growers and conducted interviews which followed the latest agreed procedures. In total, growers from 32 tomato nurseries were interviewed. This represented 186.2 hectares of crops which the TGA believe to be 98% of the UK glasshouse area currently devoted to tomato production. In fact, only one company failed to contribute to the survey. The sites ranged in size from 0.2 ha to 23.5 ha with an average of 5.8 ha. A third interim report was produced which was used by the team to tease out the key factors for inclusion in this AHDB final report. It is important to stress that several growers did not want to be identified in the AHDB report, so survey participants will be referred to by a site reference number rather than by name.

The results of the survey and associated studies will be used to prepare a proposal for further research to address the questions raised by this work.

Results and Discussion

UK tomato production statistics

There are 190 ha of commercial glasshouses devoted to long-season tomato production in the UK of which 98% were represented in the present survey. The latest available breakdown of production figures from the TGA indicate that at least 86% of UK tomatoes may be classified as ‘premium’ product rather than ‘commodity’ product with the majority of the latter being of the classic round type. The premium products comprise 31% cherry, 15% cocktail, 17% classic round and 17% mini-plum, with the remaining 6% being speciality or novelty types / cultivars.

Summary of flowering and fruit set in tomato

The term ‘fruit set’ requires definition. It is used here to denote flowers which reach anthesis (flower opening) normally and subsequently produce fruit of marketable size for that tomato type / cultivar. Very little research has been published on flowering and fruit set in tomato since the introduction of commercial bumblebees for pollination. The biological system has been very reliable and, until recently, tomato growers had very few issues with fruit set. As a consequence, most of the available literature is pre-1985.

It is useful to describe the structure of the tomato flower as this is critical to understanding the process of fruit set (Figure 1). Each flower has five or more anthers united by interlocking hairs to form a hollow cone around the style. The neck of the cone consists of sterile extensions of the anthers and these closely surround the stigma. The anthers are bi-lobed and contain several hundred pollen grains which are released through longitudinal slits. The flowers are pendant and so, when released, pollen falls onto the stigma where it should germinate. The structure should allow self-pollination though the chances of success are enhanced by any form of mechanical disturbance.

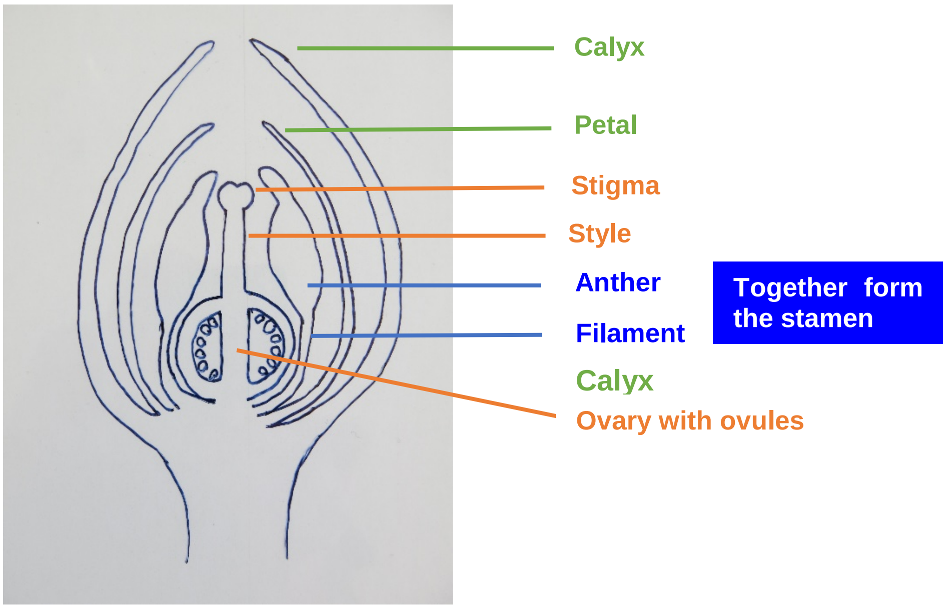

Figure 1. Diagrammatic representation of a tomato flower (NB: Female parts are shown in orange text and male parts in blue text).

Mature pollen is ready for transfer at the time of anthesis but the stigma becomes receptive about two days previously and remains so for at least four days (Smith, 1935). Once pollen grains adhere to the stigma, pollen tubes start to grow within an hour and can reach the micropyle of the ovule within 12 hours at 25°C (Dempsey, 1970). Fertilisation has been observed after 18 hours and most ovules would be fertilised within 30 hours at 20°C (Iwahori, 1966). Thus the extent of fertilisation (*i.e.* the number of fertilised ovules per ovary) is dependent on the number of viable pollen grains reaching the stigma and the effect of environmental or physiological factors on the subsequent processes of pollination and fertilisation (Picken, 1984).

Both the number and viability of pollen grains are important for successful fertilisation. Low pollen production can be caused by low assimilate supply (Howlett, 1936), high temperature

(40^{O}C) at the meiosis stage (Iwahori, 1965), or low temperature (10^{O}C) after meiosis (Maisonneuve & Philouze, 1982a). The viability of pollen can also be reduced by extreme temperatures, which adversely affect its subsequent germination. The potential number of pollen grains is genetically determined; *eg* some TMV-resistant cultivars have less pollen (Laterrot, 1971), resulting in fewer and smaller fruits than previously grown cultivars (Alexander & Oakes, 1970).

Several hundred pollen grains may be released when the anther opens 1-2 days after anthesis. Successful transfer of pollen grains to the stigma is dependent on the length of the style and for self-pollination the stigma must lie within the tip of the anther cone. The length of the style is both genetically determined (Rick & Dempsey, 1969) and affected by growing conditions (Rudich et al, 1977). The optimum position of the stigma is just within the anther. Extremes in either direction, which can be caused by poor light or high temperature, can result in poor fruit set. Pollen grains must adhere to the stigma to allow germination to take place. If the RH is below 70% or the temperature is outside the range 17-24^{O}C, the adherence of pollen may be reduced (van Ravestijn, 1970).

The number of fertilised ovules is determined by the number of germinating grains and by the successful growth of the pollen tubes reaching the reaching the micropyles of the ovules. Pollen germination is temperature dependent with the degree of germination greatly reduced outside the range of 5-37^{O}C (Dempsey, 1970).

Fertilisation takes place once the nuclei from the pollen tubes penetrate the viable ovules. It may not take place if the ovule has already deteriorated due to high temperature at the mother cell stage (*i.e.* about 9 days before anthesis). Otherwise, fertilisation is not generally affected by growing conditions.

Sub-species of Bombus terrestris

There are reported to be nine sub-species of *B. terrestris* in Europe and North Africa (Rasmont *et al.*, 2008). *Bombus terrestris terrestris* (Btt) occurs naturally in continental Europe (north of the 45th parallel) and is considered to be the ‘type species’ to which the others are compared. Three other sub-species naturally occur in mainland Europe; *B. terrestris dalmatinus* (Btd) in the south east (S.E. France to Iran), *B. terrestris lusitanicus* (Btl) in the south west (S.W. France to Maderia) and *B. terrestris calabricus* in southern Italy and Sicily. Btt, Btd and Btl have overlapping ranges with some hybridisation reported in the common territories. Four sub-species have evolved on islands; *B. terrestris audax* (Bta) in the

British Isles, *B. terrestris sassaricius* in Sardinia, *B. terrestris xanthopus* in Corsica and *B. terrestris canariensis* in the Canary Islands. Of these island sub-species, Bta is the least clearly differentiated from the type species. The ninth sub-species, *B. terrestris africanus*, is found in northern Africa.

The nine sub-species have morphological differences as well as variation in diapause, life cycle timing and colony size. In temperate climates, the queen emerges from hibernation in spring to produce a batch of non-reproducing workers. This colony of workers increases in size through the summer before consecutive batches of reproductive males and females are produced. They mate and the females become the new queens which enter diapause and hibernate through the winter. In hotter areas around the Mediterranean basin, the queens have a summer diapause usually with two generations per year (Rasmont *et al*., 2008).

It is probable that samples of most, if not all, of the nine sub-species were collected and tested in the 1980s to determine which could be reared most efficiently in culture and which would provide the best pollination of protected tomato crops. It is entirely possible that there were misidentifications at that time due to overlapping morphological features, hybridisation in the wild and assumptions based on geographical origin. Commercial breeding lines were established from the most promising samples and they have been subjected to further selection, manipulation and possible hybridisation over the years. There can be little doubt that the commercially reared bumblebees that were being released in the UK immediately prior to 2014 were very different to those originally collected or to those which were subsequently collected from the wild to supplement the breeding stock.

Perceived performance of the various Bombus terrestris sub-species

The clearest overall message from British tomato growers was the general belief that Bta are less vigorous than the previously used non-native sub-species and more likely to fail to provide adequate pollination should any aspect of flower development or ambient conditions be sub-optimal for their performance. This concept was explored in more detail in the interviews with growers and is expanded below.

The bumblebee suppliers' evaluation of Bta in the 1980s was consistent with the growers' current opinion. In fact Bta were dismissed as being inferior to Btt and Btd when populations of *B. terrestris* were originally collected and tested for commercial potential. In 2014, the three main suppliers of bumblebees in the UK made a combined response to the NE consultation robustly arguing against an enforced switch to Bta. They stated that commercially produced colonies of Bta were less efficient than the commercially reared non-native sub-species and estimated that at least 20% more hives would be required for complete pollination of tomato crops (GreatRex & Walker, pers. coms., 2014). More recently, the suppliers have been more positive about Bta; *eg* at the 2017 British Tomato Conference it was stated that there was no longer any difference between Bta and the previously used non-native sub-species (Knight, unpublished data, 2017). This change in supplier confidence in Bta may be because they have strengthened their breeding stock by selecting Bta queens which produce bigger and more vigorous colonies.

There is very little information in the scientific literature which quantifies differences in vigour between Bta, Btt and Btd. In one field experiment in southern England, paired colonies of Bta (reared from nest searching queens caught in the wild) and Btd (obtained from a commercial supplier) were found to have different nectar foraging performances, with Btd performing significantly better than Bta in four out of five study locations (Ings *et al.*, 2006). This was attributed to the larger body mass of Btd. There is a common perception among UK growers that it is the greater size of Btt and Btd which makes them more effective pollinators. In reality, the physical features of the *B. terrestris* sub-species are quite varied and there is certainly some overlap between the sub-species that have been available to British growers. For example, Figure 2 shows images of Bta and Btd queens (posted by Falk, 2013) in which the Bta queen is clearly more robust than the Btd queen.

Figure 2. Btd (left) and Bta (right) queens (Falk, 2013)

At the beginning of the grower interviews in this survey, participants were asked to score their general impression of Btt/Btd and Bta performance according to the five categories shown in Table 1. The percentage of sites that fell into each category are presented in Figure 3. These data show that 87% and 97% of growers received at least some extra Btt/Btd and Bta hives (respectively) in addition to the supplier's standard hive input programme. However, the most significant difference for the two types of bumblebees was the frequency with which those extra hives were required. For Btt/Btd, 78% of growers said they occasionally ordered extra hives and only 9% said this was a 'usual' or 'frequent' requirement. In contrast, for Bta, 28% said they occasionally ordered extra hives while 69% said this was a usual or frequent requirement.

Table 1. Bumblebee performance expressed in five categories with a score attributed to each category

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

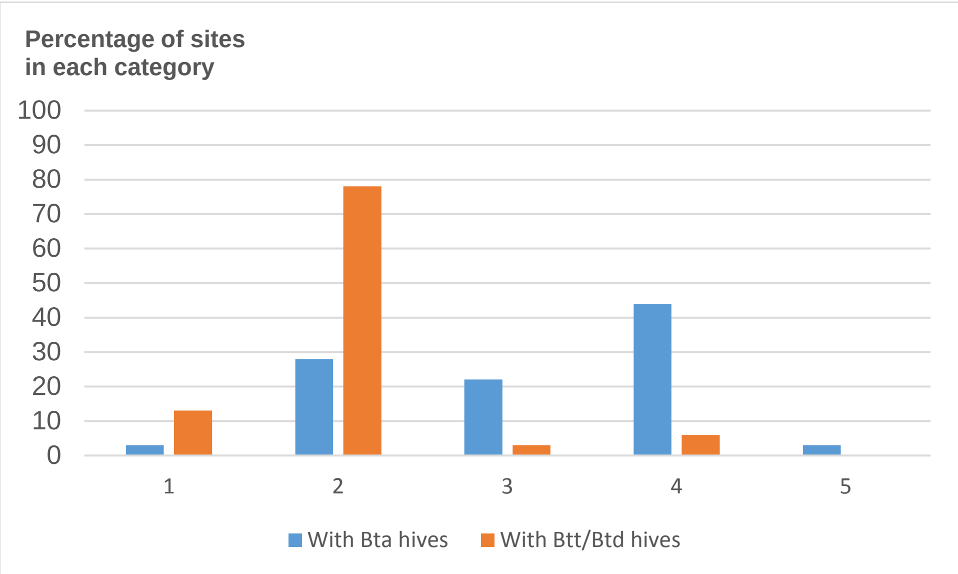

Figure 3. Growers' perception of Bta and Btt/Btd performance according to the five categories shown in Table 1 (expressed as the percentage of sites in each category).

The data shown in Figure 3 assume that each site carries equal weight regardless of the production area even though the sites varied in size from 0.2 ha to 23.5 ha. The data presented in Figure 4 take this variation into account by combining the production areas of the sites in each category and expressing those figures as a percentage of total UK production area. Exploring the production area rather than the number of sites in each performance category made small changes to the emphasis of the results but not the overall trend. For both types of bumblebees there was a small shift from the percentage in performance category 2 to performance categories 3 and 4. For Btt/Btd, there was a decline from 78% in category 2 when expressed as numbers of sites to 67.8% when expressed as production area. For Bta, there is a corresponding decline from 28% to 21.1%. This may indicate that growers with larger production areas had more issues with pollination than growers with smaller production areas but that was not a consistent rule.

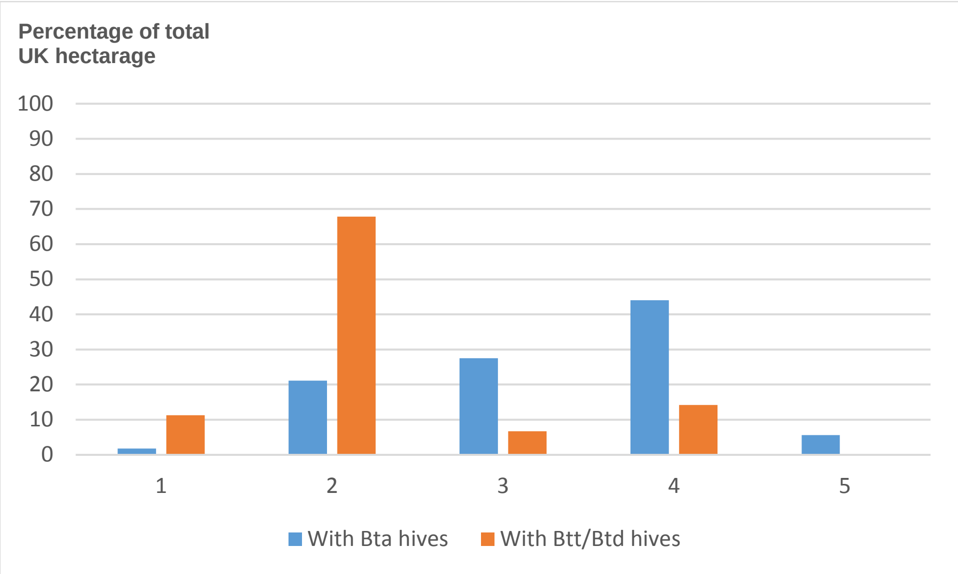

Figure 4. Growers' perception of Bta and Btt/Btd performance according to the five categories shown in Table 1 (expressed as the percentage of UK production area in each category).

It is useful to have a means of expressing each growers' perception of the performance of Bta relative to Btt/Btd. The method used was based on the scoring system shown in Table 1 with each growers' score for Btt/Btd being subtracted from their score for Bta to provide a calculated 'balance'. The seven new relative performance categories are shown in Table 2 with a brief description for each.

Table 2. Method of expressing the performance of Bta relative to Btt/Btd as perceived by individual growers

	Relative performance of Bta to Btt/Btd						
Calculated balance	-3	-2	-1	0	+1	+2	+3

Description of balance category	Verv much better	Much better	Better	Performance similar	Poorer	Much poorer	Very much poorer

The percentage of growers within each of those seven relative performance categories is shown in Figure 5. In summary, no growers considered the performance of Bta to have been better than Btt/Btd but 34% believed that the performance of the two types of bumblebees was similar. However, 28% felt that Bta was poorer, 28% said much poorer and 9% said very much poorer than Btt/Btd. Although results for Btt/Btd were by no means perfect, this chart shows that there was a strong perception among two thirds of growers that Btt/Btd provided better pollination and better fruit set than Bta.

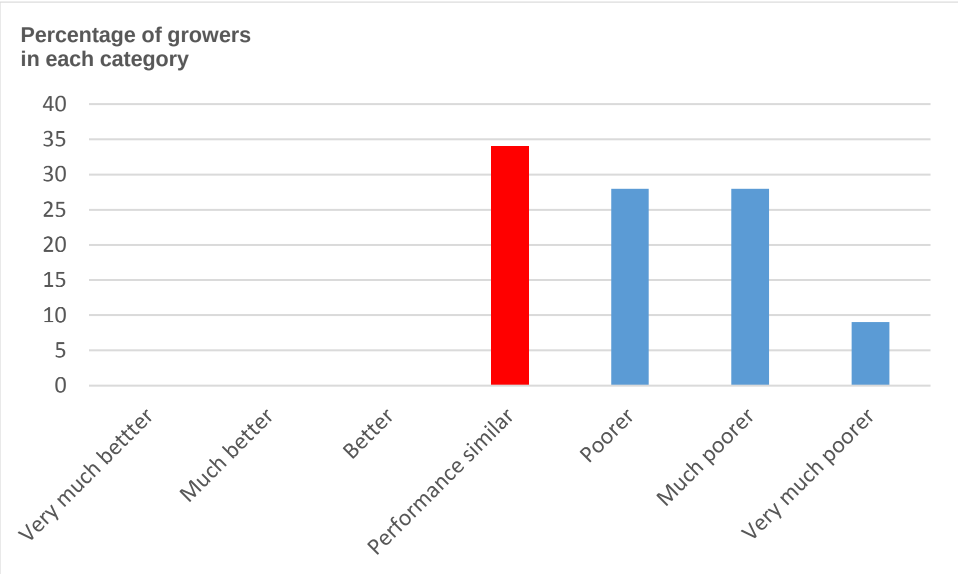

Figure 5. Growers' perception of the performance of Bta relative to Btd according to the seven relative performance categories shown in Table 2 (expressed as the percentage of growers in each category).

The data shown in Figure 5 was converted from numbers of sites into combined production areas, which resulted in a small change in emphasis but not the overall trend. In summary, no growers considered the performance of Bta to have been better than Btt/Btd but those representing 37% of the total production area felt that it was similar. However, growers representing 34% of the total production area felt that Bta was poorer, 14% said much poorer and 15% said very much poorer than Btt/Btd.

Of the 65% of growers (63% of UK production area) who firmly believed that they had experienced more issues relating to poor pollination and fruit set with Bta than previously with

Btt/Btd, only 3% provided a calculated figure for financial loss. It is difficult to express missed set in terms of financial loss because there are so many other variables which may also have an impact during the season but one grower estimated that poor fruit set cost their business approximately £50k / ha in 2015.

Due to reduced confidence in bumblebee performance, 75% of growers felt they were now devoting significantly more labour to monitoring fruit set than when they were using non-native bumblebees. However, no one had attempted to quantify the time or cost devoted to this activity. It is interesting to note that, within the other 25% of growers, approximately half had relied entirely on their suppliers to check their crops and approximately one quarter had used very large numbers of hives in their standard input schedules.

Bumblebee hive input schedules

Growers were questioned about the hive input schedules planned at the start of their growing seasons for both Bta and (in the past) Btt/Btd, as well as the frequency and number of additional hives that had been required during the seasons. It soon became clear that there were no standard input schedules. The planned schedules varied hugely between suppliers, sites, types / cultivars of tomatoes and length of the growing season. This is illustrated in Table 3 which shows examples of planned schedules for Bta hives that were produced by three different suppliers for three different sites. The data do not take into account any extra hives added to the planned schedule during the course of the season. It was interesting to note that two other growers with the smallest production areas in the survey, who grew a wide range of tomato types and sourced their bumblebees from two different suppliers, had some of the highest Bta hive input numbers (equivalent to 95 and 115 per ha) despite their shorter growing seasons.

Table 3. Examples of planned Bta hive input schedules produced by three suppliers for three different sites

	Number of Bta hives in planned input schedule				
	Grower site 1 (38 wk season)		Grower site 5 (38 wk season)		Grower site 14 (30 wk season)
	Cherry on vine	Classic on vine	Cherry on vine	Classic on vine	Classic loose
Supplier 1	87	66			
Supplier 2			114	76	
Supplier 3					Average 43

One supplier has stated that his company's Bta input schedules generally include 10-20% more hives in the standard programme than in the previous programmes that had been designed for non-native bumblebees. This was consistent with reliable data from site 17 which confirmed a 20.2% increase in hive numbers for their Bta programme. One grower of cherry and other speciality tomatoes (site 18), who obtained his bumblebees from yet another source, calculated that his hive input schedule had increased from the equivalent of 58 per ha pre-Bta to 100 per ha post-Bta.

All but one grower entered season-long contracts with their bumblebee suppliers having agreed prices for the pollination service per m^2 of tomato crop regardless of the number of hives required. Most of these growers ordered extra hives at the first indication of poor fruit set and did not keep accurate records of the total numbers of hives used during the season.

A large proportion of the growers' interviewed said they had required additional hives at some point every season regardless of the type of bumblebee. In most cases (78%), the supplier accepted the growers' judgement and provided the extra hives without further investigation. For Btt/Btd, 11% of interviewees estimated that they usually required more than one additional hive for every ten that were included in the planned schedule. However, the figure was much greater for Bta, with 79% of growers estimating that they received more than one extra for every ten planned.

Tomato types / cultivars most frequently suffering poor fruit set

All types of tomato are affected by poor set to some extent but problems are more common and more serious in the smaller fruiting cultivars which produce more flowers per truss. For example, larger fruiting cultivars, such as classic rounds, generally produce 1-2 flowers per day but small fruiting cultivars, like Piccolo and Sassari can produce 3-4 per day (Moralee, pers.com., 2017). This may be exacerbated during hot weather when Piccolo can produce 4-5 flowers per day (Udyanskyy, pers. com., 2017). Udayanskyy believes that colonies of Btt/Btd may have coped with this number of flowers but Bta are unable to do so.

The plant may compensate for missed set by producing larger remaining fruit. Assuming the larger fruit are picked loose, are sold by weight and are still within the permitted size category, then some of the lost yield will be recovered. The issues are always more serious where tomatoes of any type are ripened on the plant and harvested as whole trusses. Apart from the obvious loss of fruit, missed set within a truss results in additional work at harvest and / or in the pack house in order to make the necessary adjustments to the trusses.

One grower (site 8), who reported a marked reduction in bumblebee efficacy since the switch to Bta, also reported the move at his site to small fruiting cultivars during the same period. In 2013 *(i.e.* pre-Bta), 22% of their production area was devoted to cv Piccolo and the overall input of hives was about 60 per ha. However, by 2016 (*i.e.* post-Bta), 59% of their production area was devoted to cv Piccolo and the overall input of hives had increased to 119 per ha. This prompted the project team to look at the overall UK change to small fruiting varieties during the period that NE 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% (TGA, Unpublished data, 2016). This is clearly an important factor when considering the efficacy of bumblebees.

Some cultivars (*eg* Encore, Roterno, Sunstream) are said to be particularly vulnerable to missed set under any conditions. Several examples have been cited of such cultivars being ‘ignored’ by bumblebees when present in glasshouses containing a range of tomato types / cultivars. Several growers have suggested that this situation is now more common with Bta than it was with Btt/Btd but this has not been supported by indisputable evidence. It is important that this propensity to failed set is further investigated with plant breeders. On a positive note, it has been suggested that small plantings of cultivars such as cv Encore could be used as ‘early indicators’ of impending problems in the main crop.

Can the underlying cause of poor fruit set be attributed to plant condition?

There is a strong belief among some consultants and growers that vegetative, rather than generative, tomato plant growth results in weaker flowers and poorer set but this is still to be confirmed beyond doubt. Indeed the terms ‘vegetative’ and ‘generative’ are vague and open to different interpretation by consultants, growers and scientists. The Dutch project ‘Green Eyes’ is attempting to measure the values that growers use to determine the actions they take to manage crop growth (van Kooten, 2014). The researchers recognised that growers’ knowledge of their plants was extensive but did not necessarily connect to the language of crop physiologists. As a consequence, models developed by science could not always be used by growers and the knowledge of growers could not always be absorbed by scientists. Project Green Eyes is trying to bridge that gap and the results should be extremely helpful to studies related to flower development and fruit set. In the meantime, there are several criteria which can be used to determine whether the plant is generative. The most simple being the length of the leaf under the most recently setting truss - this varies between cultivars but in general <42cm may be considered generative. In addition, generative plants have tighter curl in the tops, thin stem diameter 20-30cm below the growing point and long thick (rather than multi-branched) roots. Factors which can contribute to more vegetative growth are:

- Excessive irrigation
- Growing in NFT
- Grafting onto certain types of rootstock
- Increasing CO_2 enrichment
- Higher temperature and / or humidity at night

Apart from some exceptional ‘one-off’ cases, which are listed below, the survey revealed little irrefutable evidence of tomato plants in the UK being outside what we traditionally consider to be acceptable vegetative / generative growth parameters. Furthermore, it would seem that modern tomato crops, which are grown in closely monitored and tightly controlled environments, would rarely be subjected to the extreme conditions that were reported in the pre-1990 studies to affect flower development. However, it is important to remember that those pre-1990 studies were done on long shelf life, classic round ‘commodity’ cultivars which were very different to the types of tomatoes now grown in the UK. It is entirely possible that the physiology of modern cultivars renders them more vulnerable to smaller variations in climatic conditions. This requires further investigation.

Six growers presented examples of exceptional cases where they believed the condition of the plants had resulted in very poor fruit set:

- Site 6 (then under different ownership) reported excessive vegetative growth in early 2014 due to restricted heat availability. This resulted in weak trusses and very poor set. Growing conditions were corrected later in the season and fruit set became normal.
- Site 13 reported excessive vegetative growth and (inferred) poor flower development in 2015 due to inappropriate growing conditions for a new cultivar (cv Terneto). The conditions were corrected during the season and the problem was resolved.
- Sites 15 and 32 claimed that root mat had caused excessive vegetative growth with a knock-on effect on flower quality, fruit set and / or fruit development.
- Site 19 described an issue with fruit set that had arisen due to excessive manganese in the water supply. The feed mix was adjusted and fruit set became normal.
- Sites 11 & 12 reported weak plants and very poor fruit set in crops in 2015 / 16 due to infection by aggressive pepino mosaic virus. Changes to crop husbandry reduced the impact of the disease in the following season and fruit set was closer to normal.

The grower at site 31 said pollen does not flow freely when the environment is too humid but he could not specify the precise conditions. He believed that Btt/Btd could cope with this reduced pollen flow but Bta were unable to do so.

Apart from the ‘one-off’ cases listed above, only 12% of growers thought that the condition of plants could have been the underlying cause of the poor set experienced during the last three years. One of those growers expressed particularly strong views (Kooijman, pers. comm., 2017). Although he believed that Bta probably are weaker than Btt/Btd, he thought that current crop husbandry practice led to poor pollen quality in modern tomato cultivars and this was the underlying cause of poor fruit set. In his opinion, if plants are strong and generative, then pollen flows freely and may not even need disturbance by bumblebees to pollinate. The latter may lead to set with negligible bruising of flowers which may be interpreted as ‘bees not working’. At his site, plants are kept strong and generative and maintained good fruit set by:

- Providing plenty of CO_2.
- Keeping vents open with pre and post night temperature dips
- Using lights whenever necessary all year round
- Constantly adjusting nutrients based on analysis.

There can be little doubt that any future studies into pollination by bumblebees must also take into account the impact of environmental conditions on the condition of plants with particular emphasis on flower development and pollen quality.

Specific feedback from growers about their experience with Bta

Where Bta seemed to have been responsible for poor set, growers were questioned about possible causal factors:

<u>Poor foraging in hot conditions:</u> 44% of growers linked poor performance of Bta to hot environmental conditions. Such conditions were poorly defined but probably involved temperatures exceeding 28^{O}C around the heads of the plants for at least 5-6 hours. This may be a direct effect of temperature on the bumblebees or an indirect effect via flower development and quality of pollen. Most of these growers based their comments on subjective observations rather than actual measurements. The remaining 56% of growers did not express an opinion on the subject.

Bta workers cooling the hive rather than foraging in hot condition: Only 47% of growers expressed an opinion on this question. They all thought that this was feasible but only 3% had attempted to measure / quantify the activity of the bumblebees. It should be noted that suppliers made changes to Bta hive design and hive placement instructions for 2017 which may help to alleviate problems related to internal overheating during hot weather in the future.

Timing of Bta activity: About one third of growers (sites 1, 3, 15, 16, 17, 20, 22, 23, 29, 30) 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 the hours that crop workers had become accustomed to seeing bumblebees working around them. One grower believed that this was because Bta forage very early in the morning so that their activity is underestimated (Theron, pers.com., 2017). If correct, then Bta activity may not be very well synchronised to pollen release and flow in flowers in glasshouse tomato crops in the UK. As yet, there is little hard evidence to support this theory but it must be investigated in more detail.

External influences: Some growers thought that at least some of their problems with fruit set had been exacerbated by the presence of pollen rich crops outside their glasshouses. For example, during oilseed rape flowering (sites 6 & 32) and when fruit trees were in blossom (site 8). However, this is not a new problem and it can be traced back to the earliest days of bumblebee use in tomato crops.

Short colony life*:* 47% of growers thought that Bta colony life was shorter than they had come to expect from non-native bumblebees. In some cases this had been acknowledged by supplier representatives (*eg* sites 1, 2, 10, 11, 12 & 29). Only a few growers had tried to quantify this and they estimated differences ranging from 2 to 6 weeks less than Btt/Btd. The remainder of growers had not monitored Bta colony life and had not received any such feedback from the supplier representatives. This does not mean that the Bta colony life was always 'normal' – just that no one had checked. Shorter than anticipated colony life could result in gaps in the planned schedule with an associated breakdown in the continuity of bumblebee activity. This might help to explain why so many additional Bta hives are ordered during the season. It was interesting to note that four growers (sites 10, 11, 12 and 31) reported improved results when they changed from a schedule based on deliveries every two weeks to deliveries every week. This presumably helped to avoid peaks and troughs of bumblebee activity.

Disrupted hive delivery schedules: During the 2014 Natural England consultation, bumblebee suppliers warned that continuity of supplies of Bta to UK growers could be difficult at certain times of the year due to the small market size and unpredictable flexibility in demand. However, no one has so far reported any difficulty in obtaining the required number of Bta hives whether as part of the original delivery programme or when ordered as extras at short notice.

Are UK growers happy to use Bta in the future or prefer to go back to Btt/Btd?:

Of the growers interviewed, 72% said they would prefer to return to using the non-native bumblebees - even where they had successfully managed Bta with additional labour input (sites 18 & 31). Only 28% said they would be happy to continue using Bta in the future.

Conclusions

- There is a general belief among growers that Bta are less vigorous than the previously used non-native sub-species and more likely to fail to provide adequate pollination should any aspect of flower development or ambient conditions be sub-optimal for their performance. 72% said they would prefer to return to using the non-native bumblebees.
- No growers considered the performance of Bta to have surpassed Btt/Btd. 28% said Bta were poorer, 28% said much poorer and 9% said very much poorer than Btt/Btd. One grower estimated that poor fruit set had cost his business £50k / ha in 2015.
- Due to reduced confidence in bumblebees, 75% of growers now devoted more labour to monitoring fruit set than when they were using non-native bumblebees.
- Planned Bta hive input schedules varied between suppliers, sites, types / cultivars of tomatoes and length of growing season. 28% of growers occasionally ordered extra Bta hives while 69% said this was a usual or frequent requirement.
- 44% of growers linked poor Bta performance to hot conditions – possibly when it was greater than 28^{O}C for 5-6 hours. This could be due to a direct effect on Bta or an indirect effect via flower quality / pollen flow.
- 47% of growers thought Bta colony life was shorter than they had come to expect from non-native bumblebees. This could result in gaps in planned hive input schedules with associated breakdowns in the continuity of bumblebee activity. Four growers reported improved results when they changed from fortnightly to weekly hive deliveries which was thought to reduce peaks and troughs in bumblebee activity.

- One grower believed that Bta workers forage very early in the morning. If correct, then their activity may not be very well synchronised to pollen release / flow in tomato flowers.
- Bta breeding stock may be becoming stronger due to producers selecting Bta queens which produce larger and more vigorous colonies.
- Only 12% of growers considered condition of plants to be the main cause of poor set.
- All types of tomato can be affected by poor set but problems are most serious in smaller fruiting cultivars which produce more flowers. It is interesting to note that the enforced switch to Bta has coincided with a national move towards small fruiting cultivars.
- One grower said pollen did not flow freely in humid conditions and speculated that Btt/Btd could cope with this but Bta could not. This should be further investigated.
- There is a strong belief among some consultants and growers that vegetative, rather than generative, tomato plant growth results in weaker flowers and poorer set. This requires further investigation.
- Future studies into fruit set should focus upon:
    - Bta biology and behaviour under different environmental conditions with particular reference to synchrony between bumblebee foraging and optimum pollen flow.
    - Bta colony life in greenhouses and the impact this has on hive input schedules and frequency of hive deliveries.
    - How environmental conditions influence flower quality and pollen flow in the small fruiting cultivars which now make up 76.9% of UK production.
    - Changes in bumblebee usage and agronomic practice that will be required to optimise all aspects of pollination and fruit set.

Knowledge and Technology Transfer

- Jacobson (2017). Reports to TGA Technical Committee meetings on;
    - 1 March 2017
    - 7 June 2017
    - 6 September 2017
    - 6 December 2017
- Jacobson (2017). Presentation at the British Tomato Conference, Kenilworth, 21 September 2017.

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