POLLINATION OF BLUEBERRY BY BUMBLEBEES

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1. INTRODUCTION

There are three types of commercially grown blueberry: (*Vaccinium australe, V. corymbosum*), lowbush (*V. angustifolium, V. myrtilloides*) and rabbiteye (*V. ashei*) (McGregor 1976). Cross-pollination of lowbush and rabbiteye blueberry plants is essential for obtaining good fruit set and yield (Free 1993). Highbush varieties are largely self-fertile, but the number of seeds per berry, fruit-set, fruit size, and speed of ripening are also increased by cross-pollination (McGregor 1976). Native bees are important pollinators for this crop, however, the native bee populations have been declining over the past several decades, due principally to bee habitat loss, and pesticide use. In addition to native bees most growers rent managed bees as a supplement (Stubbs *et al*., 2000). The use of honey bees in blueberry plants has increased significantly over the past 40 years (Stubbs *et al*., 2001). However, new challenges in the honeybee industry like increasing rates of mortality due to diseases and parasites have decreased the availability of honeybee colonies for pollination. Consequently, the rental price per colony has increased substantially. This scenario has motivated the use of alternative managed pollinators.

Bumblebees are common native pollinators of blueberry plants (*Vaccinium spp*.) (McGregor 1976). Characteristics like, higher flower handling speed (10-20 flowers/minute for bumblebees vs. 5-9 flowers/minute for honeybees) and their ability to 'buzz pollinate' (the blueberry flower needs to be vibrated to release its pollen), makes to the bumblebee a more efficient pollinator as compared to the honeybee. The Bumblebee specie *Bombus impatiens* has been developed into a highly successful commercially available pollinator for

greenhouse and open field crops. Several studies show that the bumblebees are more efficient pollinators of lowbush blueberry species as compared to honeybees (Stubbs *et al*., 2002; Stubbs and Drummond, 2001; Javorek et al., 2002) and that the use of *B. impatiens* increases the fruit weight, fruit-set and yield of this crop significantly (Stubbs and Drummond, 2001; Desjardins and De oliveira 2006).

Based on the positive experience of the use of *B. impatiens* as a pollinator on other blueberry species, several high bush blueberry growers started to introduce commercial colonies of *Bombus impatiens* for pollination. In this study we want to obtain more information about the efficiency of bumblebees as a pollinator of this crop and its qualitative and quantitative effects.

2. GOALS

- To compare the pollination efficiency of bumblebees and honeybees on blueberry
- To get information on optimum stocking rates
- To calculate the return on investment (ROI) of bumblebees in the field compared to honeybees.

3. METHODOLOGY

3.1 Sites

This study was conducted during the spring of 2006 in two locations. S*ite 1* is located in the main blueberry growing area in North Carolina. The farm where the trial took place is surrounded by forest. We conducted the trial in 4 plots of the variety Reveille. Those plots have the same management for irrigation, fertilization and pest control (Figure 1).

Site 2 is located in the main blueberry growing area in New Jersey. We conducted the trial in 3 plots of the variety Blue crop. Blueberry cultural practices were similar for all fields (Figure 2).

3.2 Treatments

High density of bumblebees

This plot was surrounded by forest and sand paths on both sites. The plots were at least 500 meters away from the closest honey bee yard.

High density of honeybees

In this treatment the honeybee yard was around 50 meter apart from both sites. For blueberry pollination the recommendation is to use between 2 to 5 hives per acre, depending on conditions that exist in the area (Stubbs *et al*., 2002). On site 1 this plot had 40 honeybee hives within 50 meters, so this plot is considered to be high honeybee density. For site 2 there were 65 honeybees' hives within 50 meters to the plot.

Bumblebees and honeybees

This scenario was evaluated only on site 1. This scenario is a combination of both treatments.

The characteristics of the plots used for every treatment on both sites are described here:

TREATMENT	CODE	SITE	PLOT	SURFACE ACRES	NUMBER OF QUAD	DENSITY IN QUAD/ACRE	TRANSECTS PER PLOT	TAGGED PLANTS	TAGGED SHOOTS
Bumblebee high density	BB1	1	1	7.76	10	1.28	6	24	72
		2	N8	9.64	12	1.25	3	15	45
Bumblebee medium density	BB2	1	1	7.76	7	0.9	6	24	72
		2	N5	18.95	8	0.42	3	15	45
Honeybee	HB	1	16	6	0	3 HIVES	6	24	72
		2	28		0	3 HIVES	3	15	45
Bumblebee + Honeybee	BB+HB	1	20, 21	8 (total)	2	QUAD 0.25 3 HIVES	6	24	72

Table 1. Treatment and Plot characteristics on both sites.

4 METHODS, RESULTS AND DISCUSSION ON POLLINATOR DENSITY AND EFFECTIVENESS

4.1 Methods

The pollinator density was evaluated trough a systematic insect survey in all the trial plots in both sites during the flowering period. Two observers recorded the number and type of flower visitors encountered, which fall into the following categories:

i. *Bombus impatiens*
ii. Honey bees
iii. Carpenter bees
iv. South western blueberry bee
v. Other visitors

On site 1 the survey consisted on recording the flower visitors present in 1 minute observation per bush. The bushes observed were distributed across the transects performed in every treatment for the fruit set evaluation and also for the bush in front of the tagged plant (see Fruit-set in methodology). The observation was done on 6 days during the flowering period (03, 04, 10, 12, 17 and 18 April 2006), number of done observations are shown in the table 2.

On site 2, each survey consisted of counting the flower visitors within a transect of 1m per ≈ 100 m long. Three transect per plot were counted, these transect were distributed randomly over the plots following the blueberry bed. Every transect was standardized to 5 minutes observation. Temperature was registered prior starting the activity observation recording. The observation were done on 4 days during the flowering period (08, 09, 10, and 11 May 2006), and number of done observations are shown in the Table 2.

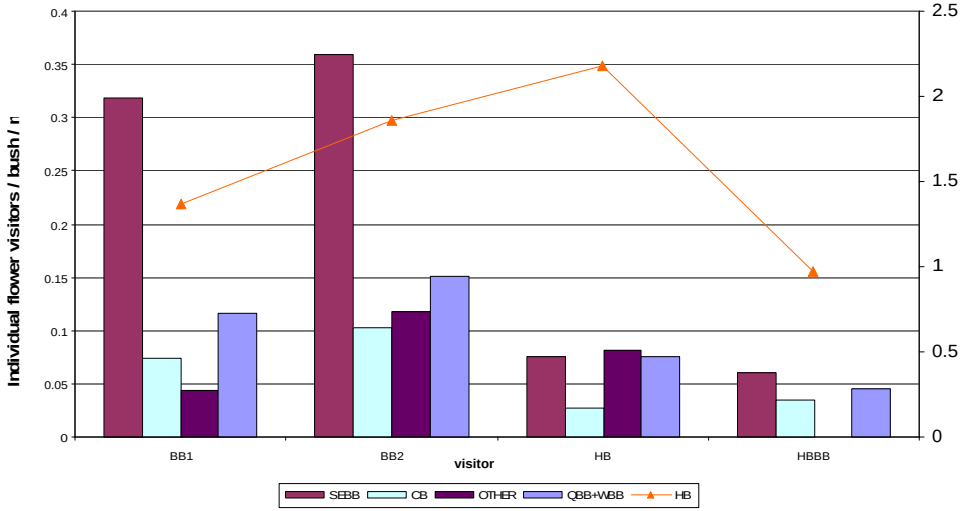
Treatment	Site	Transects (n)	Total observation time (min)
BB1	1	29	232
	2	33	165
BB2	1	33	264
	2	32	160
HB	1	23	184
	2	33	165
HBBB	1	33	264

Table 2. Total flower visiting observation time in both sites.

To estimate the efficiency of the floral visitors as pollinators, the rate of flower visit (flowers visited/minute), the frequency of stigma touch and pollen collection per visit was evaluated (Dafni, 1992). These behavior characteristics were observed and recorded in different plots randomly selected. The analysis of the rate of flower visits was done by ANOVA.

Descriptive statistics will be used to describe and analyze the data.

4.2 Results



The honeybee *Apis mellifera* was the most abundant flower visitor in both sites/varieties. The second on population density on site one was the south western blueberry bee (*Habropoda laboriosa*), this bee species is also present on site 2, but in a very low density, even difficult to observe. The Bumblebee *Bombus impatiens*, visited the flowers of both varieties of highbush blueberry bushes. The density of *B. impatiens* was higher in the treatments plots BB1 and BB2 in both sites comparing with the rest of the treatment (Figure 3 and 4).

Figure 3.Average number of flower visitor of High bush blueberry (Reveille) on four different pollination treatments in NC. The abbreviations mean: QBB Bumblebee queen (*Bombus impatiens*); WBB B. impatiens worker; SEBB south eastern blueberry bee (*Habropoda laboriosa*); HB Honey bee (*Apis mellifera*).

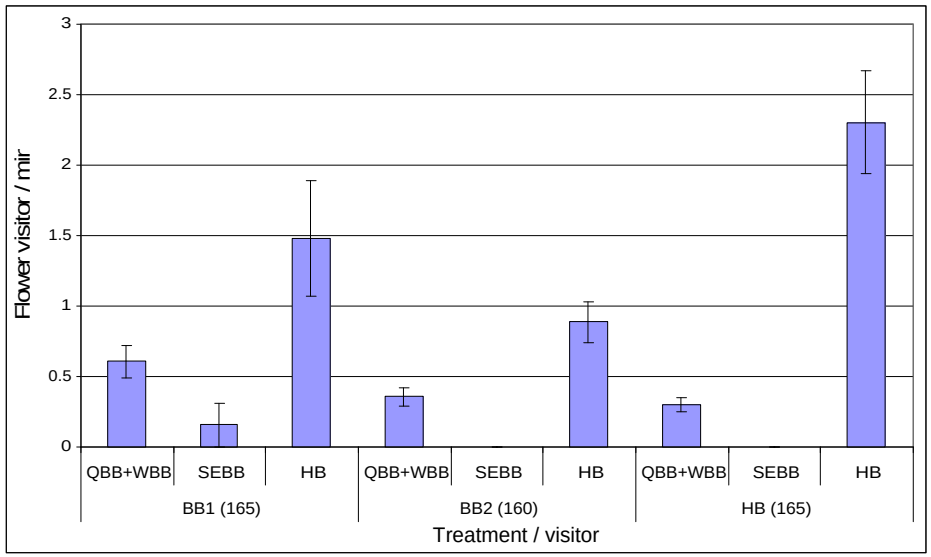


Figure 4. Average number of flower visitor of High bush blueberry (Bluecrop) on four different pollination treatments in NJ. Minutes of observation time are in parenthesis.

To evaluate the bumble bee activity as a function of the distance from the QUAD, the data from BB1 and BB2 were analyzed. There is high bumblebee activity in the first 25m decreasing with the distance from the QUAD until reach 100 m were the activity increased again (Figure 5).

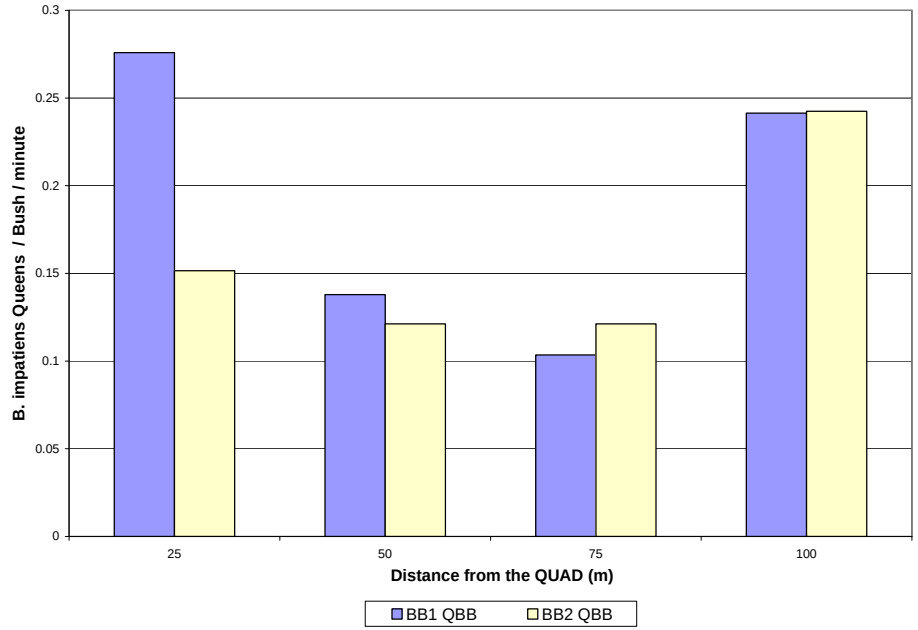


Figure 5. Average number of *Bombus impatiens* visitation flowers of High bush blueberry (Reveille) on two pollination treatment on North Carolina, USA. The abbreviations means: QBB Bumblebee queen.

The period of activity of the flower visitor differ between species. *B. impatiens* started to visit the flowers of highbush on both sites before than *A. mellifera*. On site two during the first day of observation there were not *A. mellifera* present in the crop until 12:00 next day, due to the climatic conditions (55°F minimum, 61°F maximum during day), while *B. impatiens* queens and workers were present both days since 8:00 morning (Figure 6).

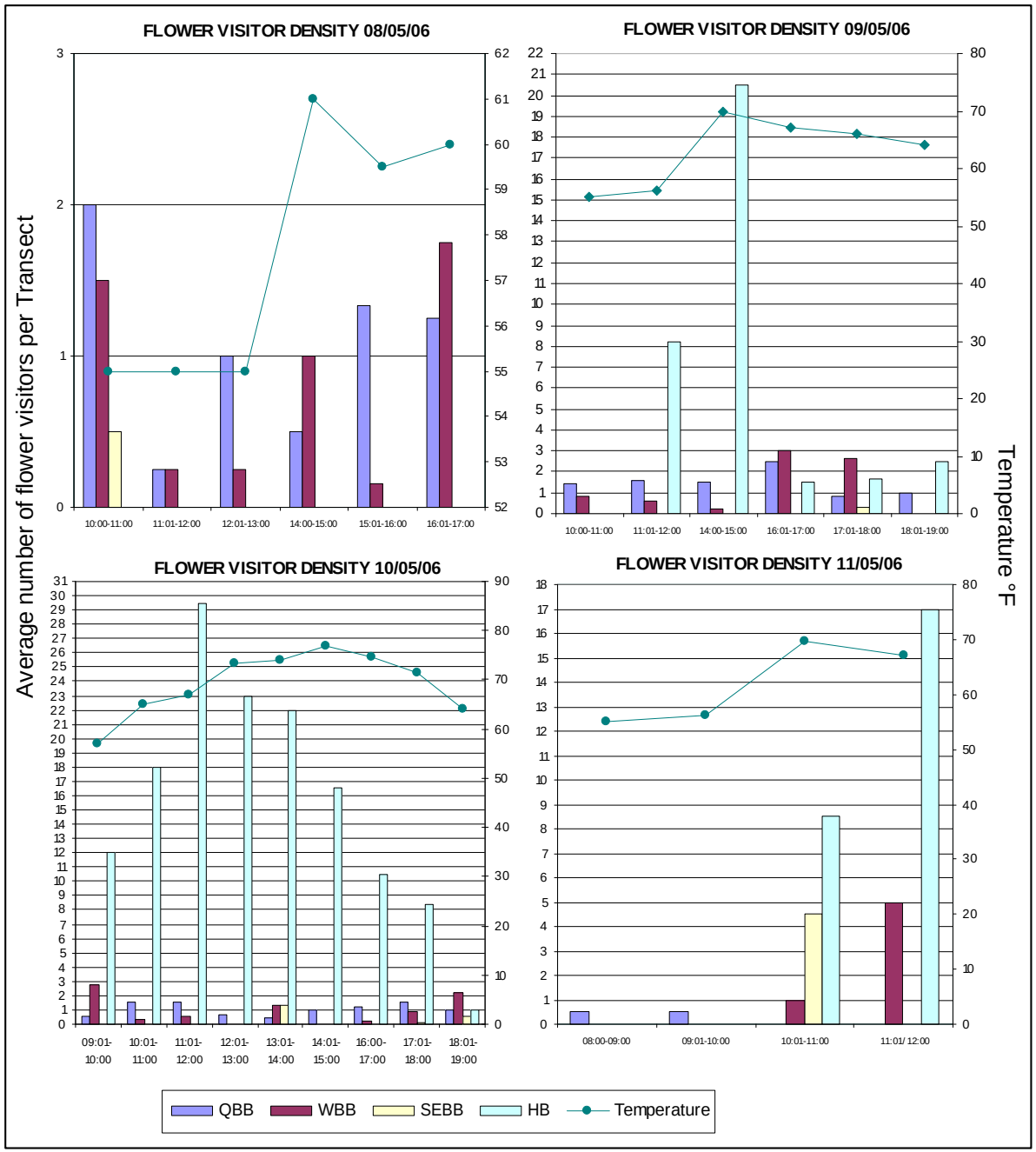


Figure 6. Average number of flower visitor of High bush blueberry (Blue crop) per transect (transects were standardized to 5 minutes observation ≈ 100 m long), on four different days during the flowering period in NJ.

On site 1 *Xylocopa virginica* and *Apis mellifera* are nectar robbers on flowers of high bush blueberry, variety Reveille. These flower visitors do not touch the stigma and they do not collect pollen frequently (Figure 7). *B. impatiens* queens and workers, the solitary bee *Habropoda laboriosa* and Andrenidae bee specie are effective pollinator of blueberry flowers, since these visitors tough the stigma and perform the pollen collection. The bee species differ significantly (df=5; F= 13.9; P=0.0001) on their rate of visit (number of flowers visited per minute) the bumblebee queen being the pollinator with higher rate of flower visit. The high rate of flower visit and their legitimate visit makes the *B. impatiens* queens the most effective blueberry pollination.

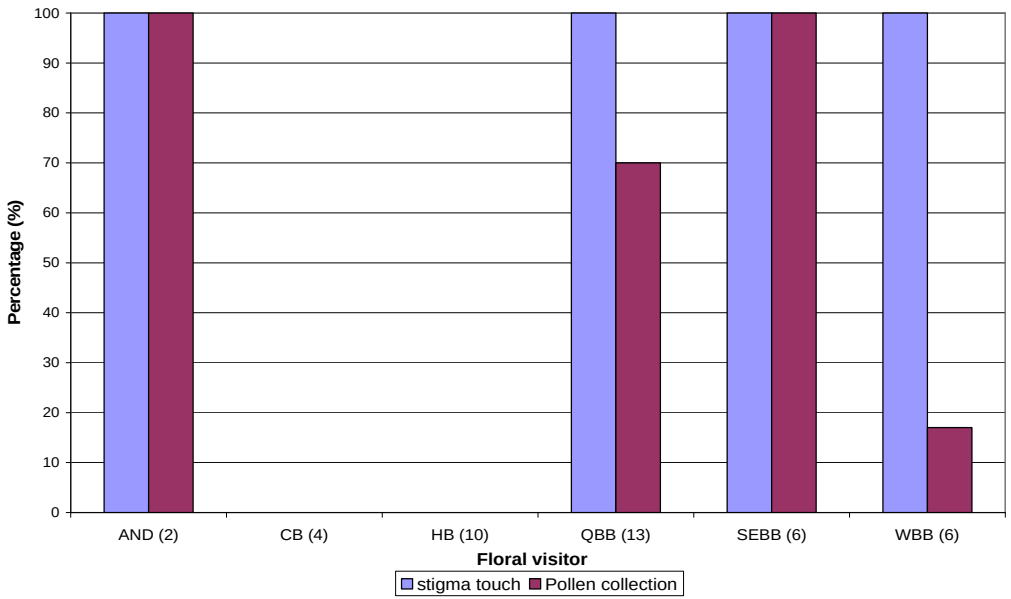


Figure 7. Pollinator behaviour. Percentage of legitimate flower visit for the flower visitors of High bush blueberry (Reveille) on North Carolina, USA. The abbreviations means: AND bee family Andrenidae; CB carpenter bee *Xilocopa virginica*; HB Honey bee *Apis mellifera*; QBB Bumblebee queen *Bombus impatiens*; SEBB south western blueberry bee *Habropoda laboriosa*; WBB worker bumble bee *B. impatiens*. Number of observation on parenthesis (n).

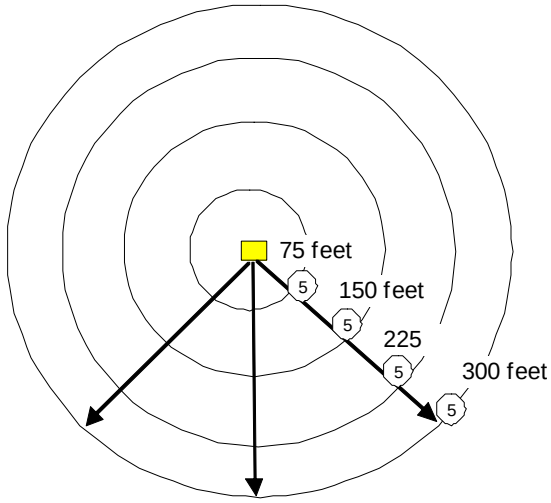
4.3 **Conclusions**

1. There is a higher pollinator diversity and density in the site one than there is in site two, where there is a high dependency of manageable pollinators due to the lack of native visitors, principally *H. laboriosa*. For growers the decision of putting in managed pollinators may be depending on the dependability of availability of native pollinators.
2. The bumblebee activity is high in the first 25 meters around the QUAD, decreasing across longer distances until reach 100 meter where the activity increase again. It suggests that a keyboard distribution pattern for the QUAD inside the crop could be the optimal distribution.
3. The most effective pollinator of blueberry flowers (highbush-Reveile) is the *B. impatiens* queen.
4. In the presence of carpenter bee (*Xilocopa virginica*), the honey bee *Apis mellifera* acts as a nectar robber on blueberry flowers, reaching the nectar trough a hole made in the corolla by the carpenter bees, pollination does not seem to take place.
5. The bumblebee activity starts early and ends later in the day as compared to other native and managed pollinators. On site two, there were a lack of any native pollinators, making bumblebees, due to bad climatic conditions, the only pollinator for almost two days during the observation period.

5 METHODS, RESULTS AND DISCUSSION ON FRUIT-SET AND YIELD.

5.1 Fruit-set.

On site 1 in order to estimate the density of *B. impatiens* at different distance from the QUADS™, and its correlation with fruit-set, six transects of 100m were established in each plot. Each transect was originated and radiated from colonies of *Bombus impatiens* QUADS™. For every transect at distances of 25, 50, 75 and 100 meters, three shoots per blueberry bushes were tagged and the number flowers recorded during blooming peak (March). See outline:



QUADS

1 2 3

The number of fruits developed in each tagged bushes were counted to determine percentage of fruit-set, dividing the number of fruits by the total number of flowers. Berries on the marked shoots were counted three weeks after the end of blooming period (5 May).

The results for every treatment were compared with descriptive (mean ± SD) and inferential statistic (ANOVA, $P \leq 0.05$) to determine if there were significant differences between treatments. For every ANOVA we used a Turkey analysis to determine which treatment differs.

5.2 Yield.

Field owners provided yield data. On site 1 we obtained the yield data from 2004 to 2006. Whereas on site 2 we used data just for the present year. Descriptive statistics analysis was used.

All the analyses were done using SPSS and JMP ver 3.1.6.2 statistics programs.

5.3 Results

5.3.1 Fruit-set.

There were significant differences in the fruit-set between the treatments in NC (ANOVA df 3; F=14.09; P=0.0001). The treatments BB1 and BB2 with higher bumblebee density had a higher fruit-set, with an average of 80.86, whereas the fruit-set in the HB treatment was of 65.89. There was a difference of 14.65% in the fruit set between the BB1 and the HB treatment. The data are shown in Figure 11 and table 3.

It should be noted that in NC the treatments with the higher densities of bumblebees coincide with higher numbers of South Eastern blueberry bee (see previous chapter). Consequently we can say that the higher fruit set is related to the presence of a ‘buzz-pollinator’

however whether the bumblebee or the South Eastern blueberry bee had the higher contribution cannot be stated based upon the available data.

In NJ there were no significant differences (DF= 2; F=2,303; P=0,10) in fruit-set due to variability of the data. The data and trends are shown in Figure 12 and table 4.

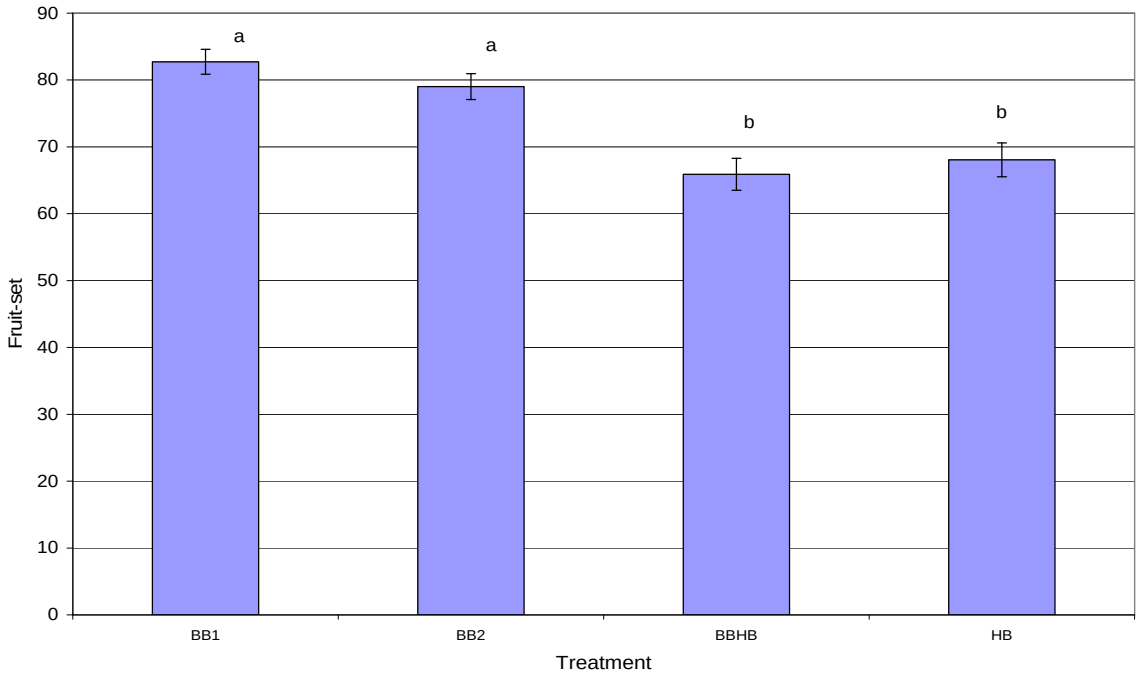


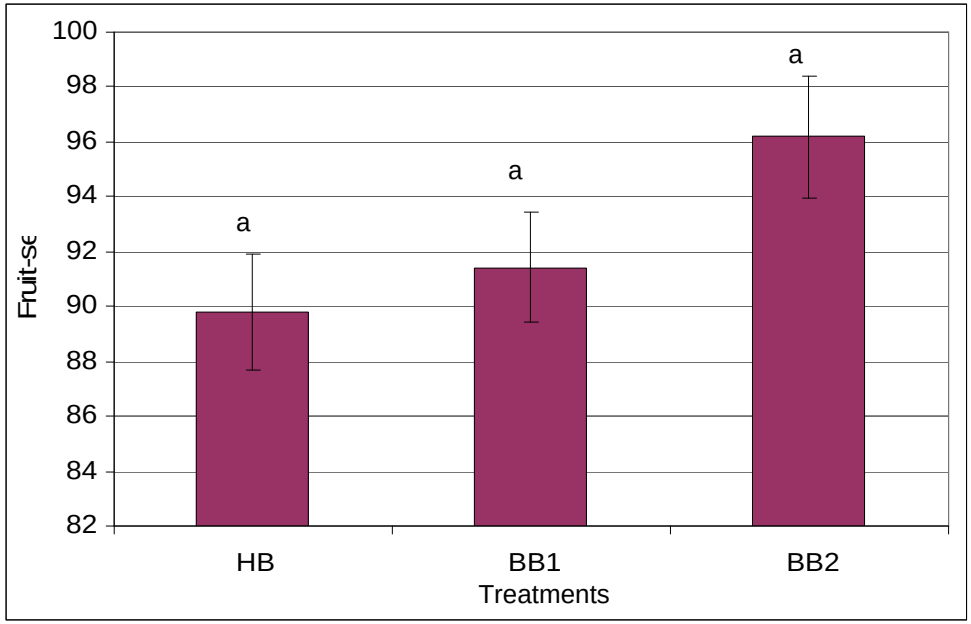
Figure 11. Fruit-set of blueberry (High bush-Reveille) bushes under different pollination treatments. Different letters means significant statistics difference (ANOVA significant level 0.05).

Descriptive Statistics

Dependent Variable: FRUITSET

TREATMEN	Mean	Std. Deviation	N
BB1	82.7191	12.7360	47
BB2	79.0188	13.3605	48
BBHB	65.8958	16.5551	48
HB	68.0622	16.9836	45
Total	73.9707	16.5055	188

Table 4. Fruit set between treatments



Treatments	(n)	Fruit-set	Std error
HB	27	89.79	2.14
BB1	30	91.43	2.03
BB2	25	96.18	2.22

Figure 12. Fruit-set of blueberry (Bluecrop) bushes under different pollination treatments. Different letters means significant statistics difference (ANOVA significant level 0.05).

5.3.2 Yield.

Site 1, NC. The final yields are represented in table 6. Berries harvested are indexed where the honeybee treatment represents the standard (100). Grower was unable to provide data for the 2 bumblebee treatments separately. Treatments BB1 and BB2 were harvested together and averaged in table 6. Histocal data for the plots showed great variance between plots.

	YIELD/ACRE
BB	123
HB	100
HB+BB	113

Site 2, NJ. The yield data (quality and quantity of the fruits) are shown in figure 13.

The fruits from the two treatments with bumblebees (N5 and N8) had a bigger weight than the ones collected from HB treatment. The fruit quality named as “Total score” from the farm owner was also superior from the fruits collected on bumblebee treatments. According to yield data from 2005 and 2006 years, there is a increase of 39% in the yield obtained on plot N8 (high bumblebee density BB1) between years, while on plot 28 (HB honey bee treatment) the annual increase was only 5% (Figure 13).

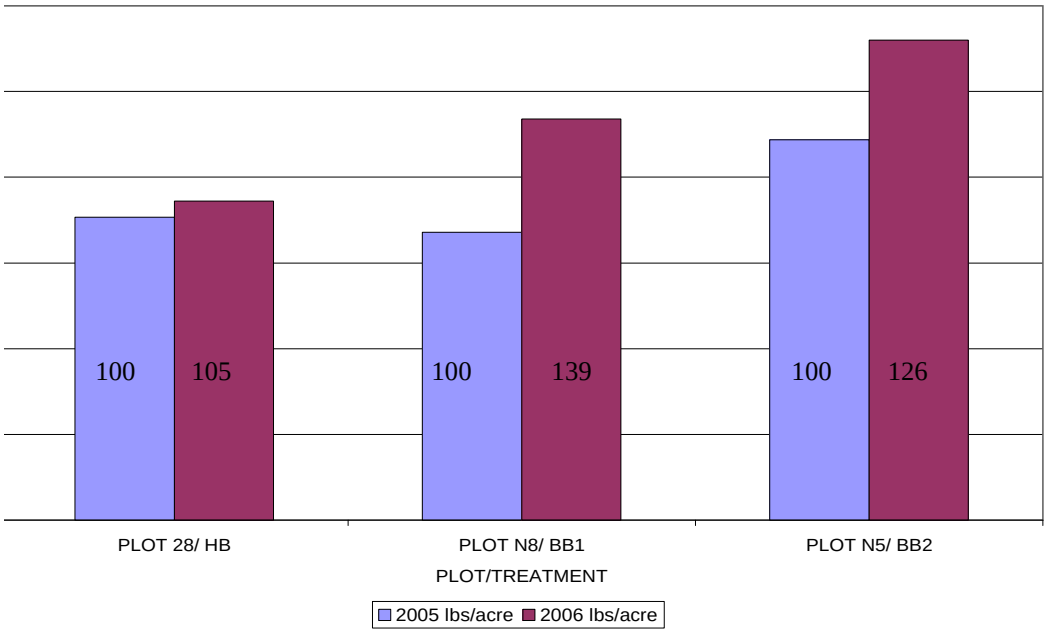


Figure 13. Comparison of the yield in three plots under different pollination management in blueberry (Bluecrop). The data are the total average of the annual yield indexed with 2005 set at 100.

5.4 Return on Investment

On site 1; NC the yield on the bumblebee plot was 23% higher as compared to the standard (honeybees). Assuming an average yield of 5000 lbs./acre this would translate in 1150 lbs. of extra berries.

On site 2; NJ the yield on the bumblebee plot was 26% higher as compared to the standard. With the same assumption as above this would translate in 1300 lbs. of extra berries.

Currently the highest price for a QUAD (4 hives) is $ 225,-. The highest shipping cost are $ 20,-/QUAD. This makes the investment per acre at the recommended rate (3 hives/acre) $ 185,- at most. Assuming an average price of $ 1.25/ lbs. of berries the extra yield that needs to be obtained to break even on the bumblebee investment needs to be 150 lbs./acre at most. This is comparatively speaking to no pollination investment.

Compared to a honeybee investment the extra yield that needs to be obtained is depending on local honeybee hive prices and is 50 lbs. of extra berries per acre at a honeybee hive price of $ 35,-. At $ 55 - $ 60,- per honeybee hive the investment in pollination in bumblebees equals that of honeybees and in areas where honeybee hives cost more than 65,-/hive bumblebee are a cheaper alternative when put out at the same rate (in hives/acre).

Pollination needs to be viewed as a means to improve crop potential. Yield is obviously influenced by pollination, however other factors come into play when assessing yield as well. Fruit set is the best measure to evaluate the success of pollination, whether each fruit that is set actually translates into a harvestable berry depends on many more factors besides pollination and the used pollinator.

5.5 Conclusions

6. Improving the bumblebee density through the introduction of QUADS had a positive effect on the fruit-set and yield of the blueberry fruit in both Blueberry varieties.
7. The fruit-set in Reveille blueberry bushes on plots with high density of Bumblebees increased on 14%, although it is not clear yet how much of this positive effect was due only by the bumblebee pollination.
8. On site 2 there was an increase in the weight per berry (count per pound) of 31% on the first pick and 4% on the second one, there were also an increase of 39% on yield compared with the previous year.
9. Return on investment in plot 1 was $ 7.77 dollars returned per dollar invested. In plot 2 it was $ 10.11 dollars returned per dollar invested.
10. The use of bumblebees as a managed pollinator of highbush blueberry crops is an effective alternative to honeybees for farmers.

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