

# Evaluating the Effect of Honey Bee Stocking Density on Bee Growth and Fruit Development in Wild Blueberry

Atlantic Tech Transfer Team for Apiculture (ATTTA) 2017

## Abstract

Wild blueberry (*Vaccinium angustifolium* Aiton) is a major crop in eastern Canada that depends on insect pollination. The majority of pollination is carried out by bees. Blueberry producers typically rent honey bee (*Apis mellifera* L.) colonies, and/or other managed bees, in order to facilitate cross-pollination.

We investigated colony stocking density during pollination to facilitate optimized berry yield, while also observing the effects of hive density on bee health before and after pollination. We found that the total number of berries per stem at harvest (August) was highest in fields with 5.1 honey bee hives per acre, although this was not significantly different from fields with 3 hives per acre mixed with 1 bumble bee quad per acre or fields with 2 hives per acre. Average berry mass was significantly highest in fields with no managed bees. Colonies stocked at 3 hives per acre expanded the most, as measured by seams of bees, but growth was not significantly higher than bees stocked at 1 or 2 hives per acre.

We conclude that stocking honey bee hives at 1 and 1.5 hives per acre is not adequate for optimal fruit yields, and that by stocking at 3 hives per acre with bumble bees, or 5.1 hives per acre, almost twice as many berries can be produced per stem. Further study is required to determine the impact of weather (e.g. rainfall, temperature during pollination), fertility, crop protection agent timing and pest pressure, colony strength during pollination, pollen deposition on stigmas, pollinator interactions (e.g. bumble bee quads, honey bees, native bees), and previous harvester type on wild blueberry production. The upper limit of stocking density remains to be determined.

## Introduction

Wild blueberry (*Vaccinium angustifolium* Aiton), a significant crop in eastern Canada and the northeastern United States, depends on insect pollination that is primarily carried out by bees. Blueberry growers often used commercially managed bees, particularly the honey bee (*Apis mellifera* Linnaeus), to achieve adequate cross-pollination and subsequent fruit set. There is currently a wide variance in the stocking density of honey bees used in wild blueberry pollination in Atlantic Canada, and differences exist even among fields on the same farm depending upon field history, plant cover, and availability and pricing of commercial pollinators (Eaton and Nams 2012). Determination of the most appropriate honey bee stocking density during wild blueberry pollination is important for efficient resource use, maximizing production, and overall advancement of the wild blueberry industry. Current recommendations for honey

### Funders and Contributors:

Bleuets NB Blueberries

Nova Scotia Beekeepers' Association

Prince Edward Island Wild Blueberry Growers Association

Oxford Frozen Foods

New Brunswick Beekeepers Association Inc.

Wild Blueberry Producers' Association of Nova Scotia

PEI Beekeepers' Association

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bee stocking densities vary. Past recommendations have included 2.5-5 hives per ha (1-2 hives per acre), although in some regions, particularly in large fields, up to 12 hives/ha (5 hives per acre) are used (Savoie and Argall 1996; Drummond 2002). A large-scale field study demonstrated that linear blueberry yield increases in Nova Scotia were only assured up to 4 honey bee hives/ha (1.6 hives per acre) (Eaton and Nams 2012). In some cases, sufficient wild bee abundance resulted in negligible effect of honey bee hives on fruit set and yield (Eaton and Nams 2012). In New Brunswick's 2013-2018 Wild Blueberry Sector Strategy Report, however, increasing pollination capacity to 3 honey bee hives per acre is clearly outlined (Province of New Brunswick undated). This identifies the need for honey bees as a 'pollinator insurance policy' due to the variability of wild bee populations (Drummond 2002; Eaton and Nams 2012). By considering bee stocking density, recommendations for pollinating wild blueberry can be developed and adapted to individual circumstances and fields.

Seeing as there are many factors that impact wild blueberry yield beyond pollination including plant genetics, soil fertility, disease and pest pressures, weather conditions, and harvest equipment, we monitored fruit set, retention and mass throughout the season to pinpoint if pollination was optimized and if other factors impacted blueberry yields. For example, if there was excellent pollination and fruit set, but berries dropped during the summer, other factors were probably involved (perhaps disease pressure, or the plant could not support all the berries) (Chiasson and Argall 1996b; Drummond 2002; Melathopoulos 2015). This information can provide guidance to producers as they are making management decisions.

Although increased blueberry production due to improved pollination was outlined as an opportunity in New Brunswick's Wild Blueberry Sector Strategy, inadequate access to reliable, healthy and strong managed pollinators was identified as a threat to the industry (Province of New Brunswick undated). To address this, we monitored honey bee health during blueberry bloom by tracking colony strength via seams of bees. Wild blueberry flowers provide limited amounts of pollen and nectar for bees (Chiasson and Argall 1996b; Drummond 2002), and the pollen does not provide adequate crude protein to honey bees (Somerville 2000, Somerville 2001). This could impact overall colony nutrition with potential for serious consequences, including reduced lifespans, foraging activity and larval production (Brod-schneider and Crailsheim 2010). In this project, we only focused on the strength of managed bees while in blueberry fields.

Our objectives were to determine the ideal bee stocking density to optimize fruit set and yield of wild blueberry, and to monitor honey bee colony growth before and after wild blueberry bloom. We hypothesized that higher stocking densities would result in more berries per stem and greater berry mass. We also predicted that honey bee colony growth would slow or regress at higher stocking densities, due to competition for floral resources and limited foraging opportunities.

## Materials and Methods

A completely randomized design was used with one factor (honey bee hive density) and seven levels: 0 hives, 1 hive per acre, 1.5 hives per acre, 2 hives per acre, 3 hives per acre, 3 hives per acre + 1 bumble bee quad per acre, and 5.1 hives per acre. Due to the anticipated lower price in 2017, only one field at the 5.1 hives per acre density could be found to test in northeastern New Brunswick. All colonies were managed similarly, with at least two boxes (e.g. two brood chambers or one brood chamber, one honey super). The study was carried out in Gloucester, Northumberland, and Kent counties in New Brunswick (hereafter referred to as 'northeastern NB') (Figure 1).

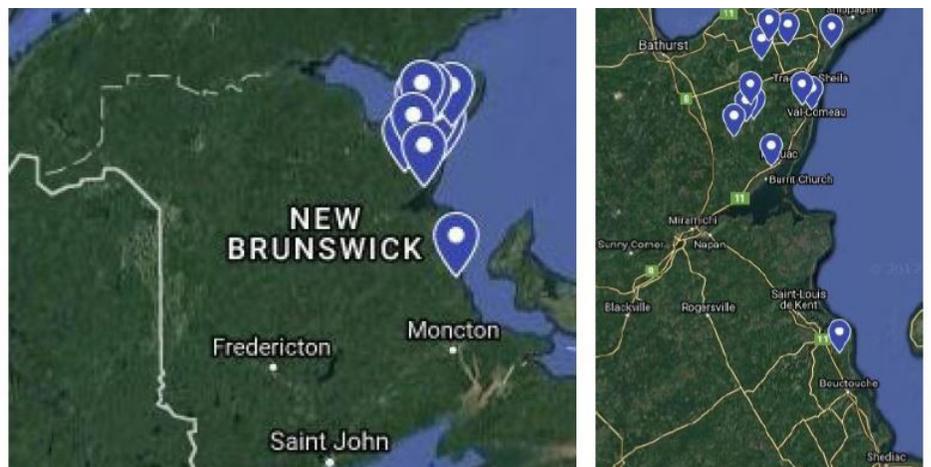


Figure 1. Map of field site locations for pollination study in New Brunswick, 2017.

### Field selection

Growers were selected in northeastern NB based on their isolation (isolated from other study sites by at least 3 km). Fields were less than 10 acres (~ 4 ha) in area and were mature (in production for at least 8 years). Permission was granted from individual beekeepers to assess colony strength.

Twelve fields were used throughout the study (Table 1). Additional fields were included in the beginning, but were later removed due to small hive beetle (SHB) (*Aethina tumida*) findings. Unfortunately, many of these fields contained higher stocking densities of honey bees, potentially creating a clearer picture of the upper limit of stocking density for pollination.

Table 1. Study fields used in the pollination trial in New Brunswick, 2017.

<b>Stocking Density (hives per acre)</b>	<b>Location</b>	<b>County</b>	<b>GPS Coordinates</b>
0	Neguac	Northumberland	47°14'27.3"N 65°06'48.4"W
0	Val Comeau	Northumberland	47°26'07.1"N 64°54'37.7"W
0	Range 1	Gloucester	47°23'46.1"N 65°14'33.5"W
0	Range 2	Gloucester	47°24'07.8"N 65°12'17.1"W
1	Inkerman	Gloucester	47°38'41.5"N 64°48'58.0"W
1	Pte. a Tom	Gloucester	47°27'07.9"N 64°57'51.4"W
1.5	Paquetville	Gloucester	47°39'52.8"N 65°07'41.3"W
2	Landry	Gloucester	47°39'05.2"N 65°02'05.8"W
3	Richibucto	Kent	46°36'24.3"N 64°46'27.5"W
3	B11	Northumberland	47°20'26.9"N 65°18'08.4"W
3 + 1 bumble bee quad	B14	Northumberland	47°19'59.6"N 65°16'50.7"W
5.1	Val Doucet	Gloucester	47°36'02.3"N 65°09'51.1"W

### Bee Sampling

Colony strength was quantified at the beginning and end of blueberry bloom by recording the number of seams of bees. The first sampling period occurred within three days of the hives being placed in blueberry fields, and the second sampling occurred within three days of the colonies being removed from blueberry fields. The hives studied were in their first blueberry pollination to reduce variability.

### Blueberry Sampling

Fifty stems within each study field were randomly selected before flowering (early May) by walking slowly through the fields in a zig-zag pattern (Chiasson and Argall 1996a; Drummond 2002). Each stem was tagged with flagging tape with a corresponding sample number in order to track number of flowers (May), fruit set (June), and harvest (August).

### Statistics

The model assumptions of normal distribution and constant variance of the residuals were met for bee growth and number of flowers per stem in May, and analysis of variance (ANOVA) was used to detect differences among mean number of seams of bees and mean number of flowers per stem, respectively. The assumptions could not be met for mean berries per stem in June or at harvest, nor mean berry mass at harvest, so a cube root transformation was applied to both mean berries per stem parameters, and a square root transformation was used for mean berry mass at harvest. All means were back transformed for reporting. Multiple means comparisons were conducted



Figure 2. Evaluating selected wild blueberry stems

using Tukey's test to compare the least squares means for significant effects. Letter groupings (pdmix macro) were produced to show significant differences among means using  $\alpha = 0.05$ . All statistical analyses were carried out using the Mixed Procedure in SAS v. 9.4 (SAS Institute Inc. 2014).

### Results

The number of flowers per stem in each study field in May was relatively consistent at approximately 40 flowers per stem, with only the sites at 3 hives per acre having slightly less flowers per stem (Figure 4). The consistency of flowers per stem among sample fields indicated the fields were fairly uniform and had similar fruit production potential.



Figure 3. Progression of wild blueberry development in experimental fields in New Brunswick (bloom > fruit set > nearly ready for harvest).

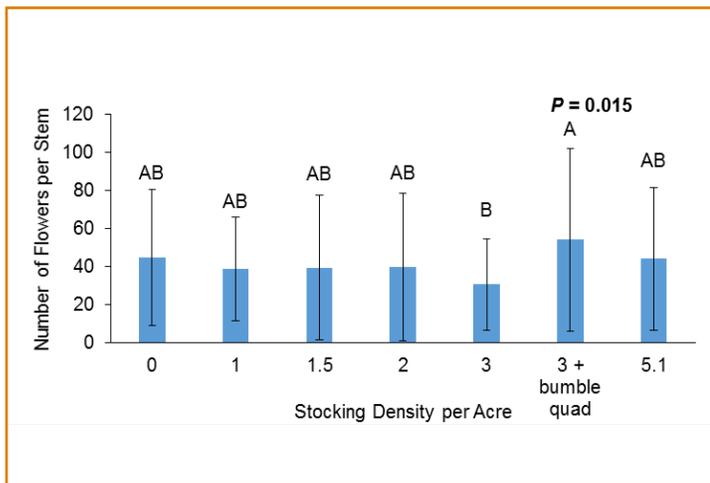


Figure 4. Mean ( $\pm$  standard deviation) number of flowers per stem in May in wild blueberry fields with varying pollinator stocking densities in northeastern New Brunswick, 2017. Means that do not share the same letter are significantly different at the 5% level.

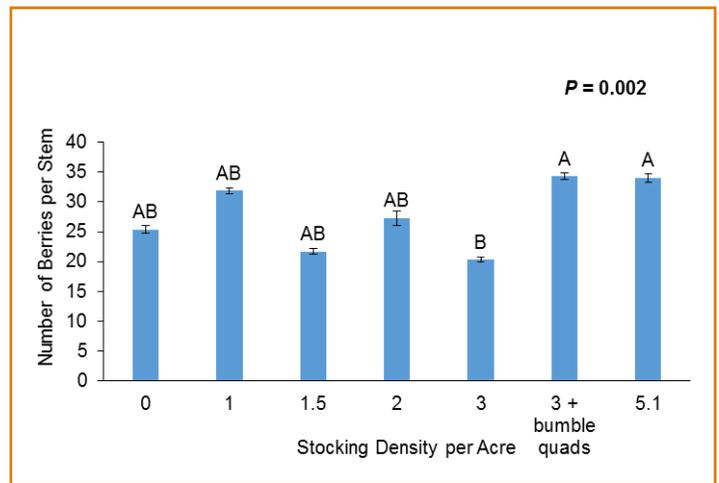


Figure 5. Mean ( $\pm$  standard deviation) number of berries per stem in June in wild blueberry fields with varying pollinator stocking densities in northeastern New Brunswick, 2017. Means that do not share the same letter are significantly different at the 5% level.

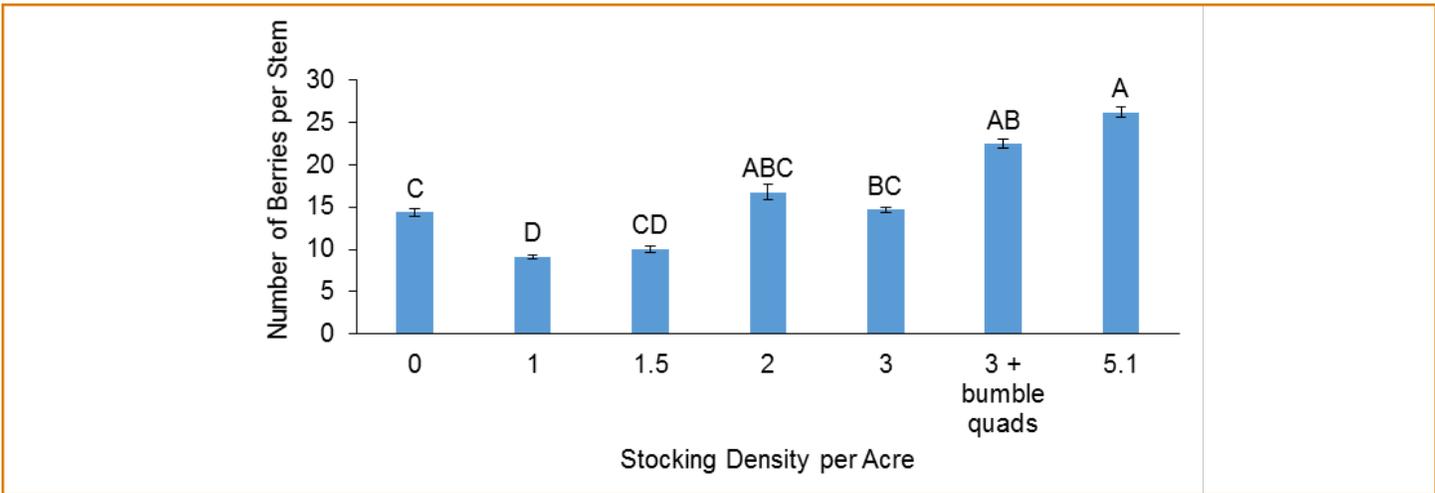


Figure 6. Mean ( $\pm$  standard deviation) number of berries per stem at harvest (August) in wild blueberry fields with varying pollinator stocking densities in northeastern New Brunswick, 2017. Means that do not share the same letter are significantly different at the 5% level.

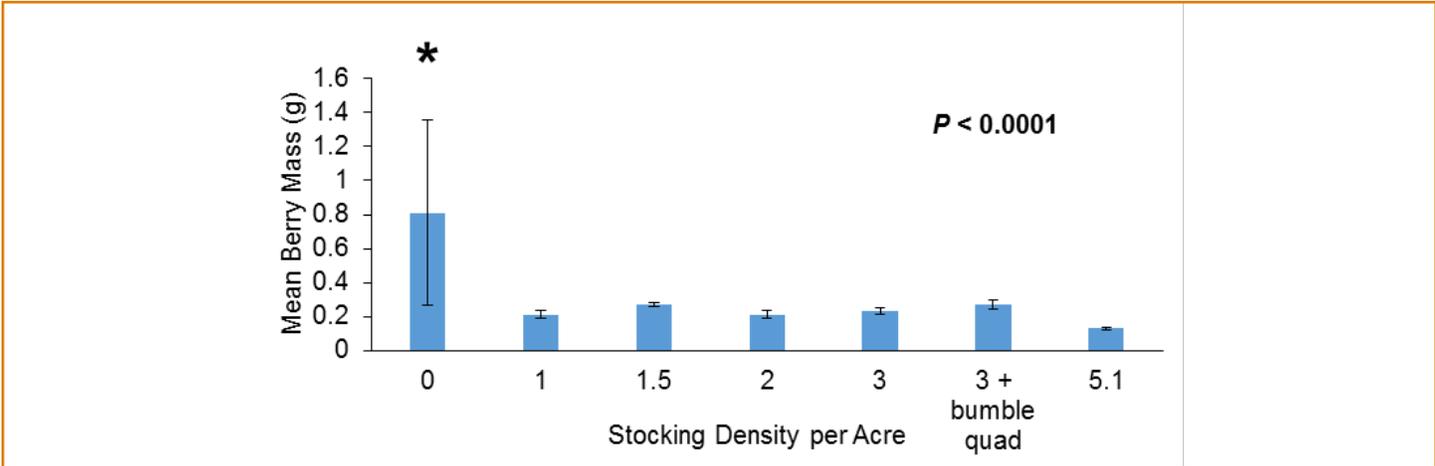


Figure 7. Mean ( $\pm$  standard deviation) berry mass at harvest (August) in wild blueberry fields with varying pollinator stocking densities in northeastern New Brunswick, 2017. Means with an asterisk are significantly different at the 5% level.

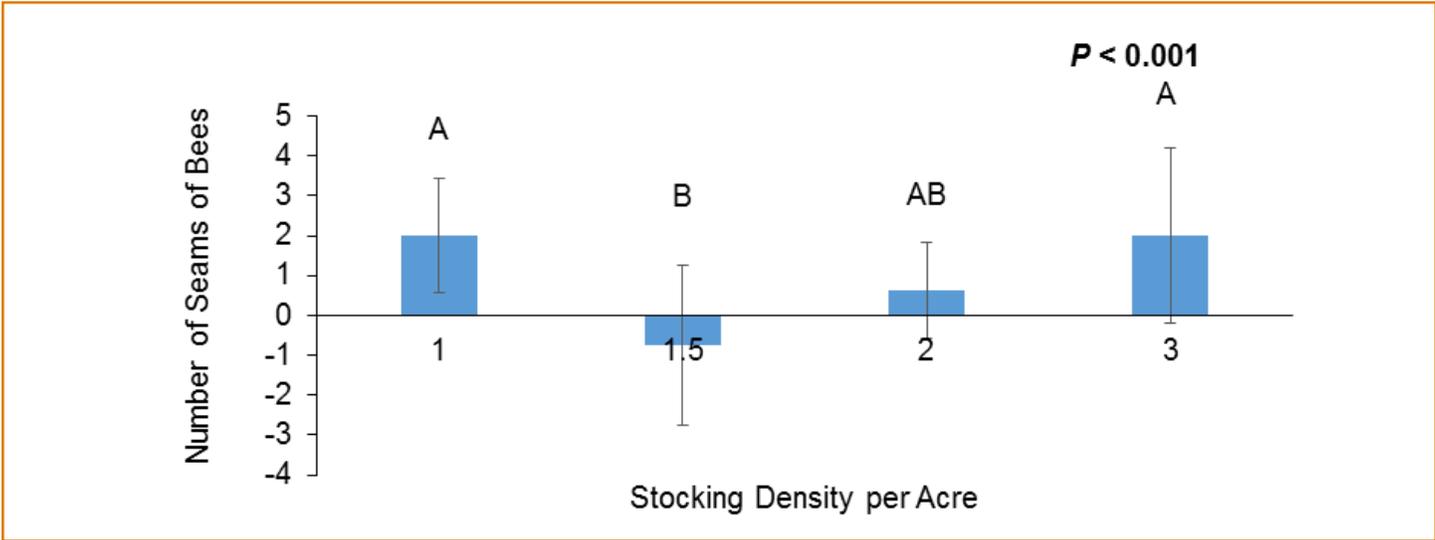


Figure 8. Mean ( $\pm$  standard deviation) bee growth (or decline) as measured by number of seams of bees within a honey bee hive during pollination in wild blueberry fields with varying pollinator stocking densities in northeastern New Brunswick, 2017. Means that do not share the same letter are significantly different at the 5% level.

## Discussion

Our hypothesis that higher stocking densities would result in more berries per stem was partially fulfilled. As stocking density increased, the number of berries per stem generally increased, although fields with 0 hives per acre did not result in a significantly different number of berries per stem compared to 3 hives per acre. There were more berries per stem in fields with 5.1 hives per acre, but not significantly more than in fields with 2 hives per acre or 3 hives per acre plus one bumble bee quad per acre.

Berries had significantly higher mass in fields with 0 honey bee hives per acre, contrary to our expectations. This may be because native bees are known to be more efficient and effective pollinators of lowbush blueberry (e.g. Javorek et al. 2002; Drummond 2016, Asare et al. 2017).

We predicted that honey bee colony growth would regress at higher stocking densities, but this was not realized. Hives stocked at 1 hive per acre and 3 hives per acre displayed greatest growth (as measured by number of seams of bees), although not significantly more than stocking densities of 2 hives per acre. Unfortunately, the hives at the 5.1 hives per acre stocking density could not be assessed due to SHB findings.

Certain fields contained hives that did not reach the pollination standard and were weak before blueberry pollination, potentially contributing to their slow growth or even regression of number of seams of bees. The pollination standard according to the New Brunswick Department of Agriculture (Savoie and Argall 1996) is:

- At least two boxes or supers
- A laying queen
- Brood
- 25,000 to 30,000 bees

In comparison, the pollination standard according to the Nova Scotia Beekeeper's Association is:

- 4 frames of brood with 100% brood coverage (or equivalent)
- 8 frames of bees with 100% bee coverage (or equivalent)
- 2 frames of honey
- 1 laying queen

Eaton et al. (2004) recommended a similar standard, including 20,000 worker bees, one young productive queen, four full frames of brood, and two full frames of honey and pollen. Contrary to some weak colonies observed, many hives from certain beekeepers exceeded the pollination standard and expanded significantly. Basic management practices pertaining to spring build up and pollination preparation may be advantageous for certain beekeepers in this region. However, the exceptional beekeeping skills and high-quality hives available from New Brunswick beekeepers in time for wild blueberry pollination should be recognized and considered.

This trial was performed during one production season and in one specific region; therefore limited conclusions can be formed at this time. Certain stocking densities had low replication due to extenuating circumstances (e.g. 1.5 hives per acre, 5.1 hives per acre). The predicted low price of wild blueberries hindered some producers from stocking fields at their typical densities, preventing us from determining the upper limit of stocking density on yield. The variability of native pollinators at each site is unknown but likely contributed to pollination success. Quantifying the presence of native pollinators during pollination is important moving forward.

The drought-like conditions of the 2017 growing season likely influenced our results and potentially masked the impacts of different stocking densities on fruit yield and berry mass. This was particularly evident between fruit set in June and total berries per stem in August, for example, the number of berries per stem decreased in all fields between June and August. We recommend this trial be replicated across multiple growing seasons in order to test these questions under different weather conditions.

We expected a linear increase in berry yield as stocking density increased, to approximately 4 hives per hectare (Eaton and Nams 2012) (~1.6 hives per acre), but we found a fairly linear increase to at least 5.1 hives per acre. There were replicate fields stocked with 3 hives per acre that resulted in lower yields than other fields stocked with a lower hive density. Some of the fields at this stocking density began the season with fewer flowers per stem (although not significantly fewer), reducing their yield potential. Additionally, several of the 0 hive per acre fields yielded higher than fields with honey bee hives, potentially due to the presence of native bees, healthier plants, reduced pest and disease pressure, harvester type, or climatic conditions.

There is anecdotal evidence for the benefits of mixing honey bee hives and bumble bee quads in blueberry fields for higher yields and our results seem to support this hypothesis. Complementary pollination from managed honey bees and other pollinators has been reported in other agricultural crops, such as sunflowers (Greenleaf and Kremen 2006), soybeans (Milfont et al. 2013), and pumpkins (Hoehn et al. 2008). It may be beneficial to continue research efforts into optimizing complementary pollination services.

We are unsure why bees in certain fields declined in strength, but it is likely linked to their weak start. These hives may not have had the pollination workforce to gather resources and grow, especially with the additional stress of being moved to fields for pollination. More work is required to understand the transition from pollen collection to nectar foraging. Imported hives from some of the test fields contained SHB, causing some local beekeepers to remove their hives from fields prematurely, which potentially impacted pollination success.

We do not know the impact of harvester type during the previous harvest season on plant structure or health. It is possible that with highly mechanized harvesting machines, the plants may be roughly handled, potentially negatively impacting future production. Stem density did not vary greatly among the tested fields, but is expected to vary among clones and among fields of different histories and years of establishment. Including stem density as a covariate moving forward would likely be valuable. The plant health status was unknown in the test fields, as was the fertility and crop protection (pesticides) programs. These key management tactics conceivably impacted fruit development. Pest pressures were not quantified in the test fields, but this variable may have impacted fruit development.

Our preliminary results demonstrate that with the exception of certain fields, 1 and 1.5 hives per acre is not sufficient to maximize fruit yields. With 3 hives per acre plus one bumble bee quad per acre, or alternatively with 5.1 hives per acre, producers could double the number of berries per stem. We recommend stigma collection in future studies in order to evaluate *Vaccinium* pollen deposits. Research remains to be done on optimal hive placement, including where in the field (e.g. field edge, in centre of field), when (e.g. 0 % bloom versus 25 % bloom), and at which hive strength. The upper limit of stocking density is yet to be determined, as well as the holding capacity of plants.

## **Conclusion**

We found that the total number of berries per stem was highest at harvest (August) in fields with 5.1 honey bee hives per acre, although this was not significantly different from fields with 3 hives per acre mixed with 1 bumble bee quad per acre, nor 2 hives per acre. Mean berry mass was significantly highest in fields with no managed bees. Colonies stocked at 3 hives per acre expanded the most, as measured by seams of bees, but did not grow significantly more than colonies at 1 hive per acre nor 2 hives per acre. We conclude that stocking honey bee hives at 1 and 1.5 hives per acre is not adequate for optimal fruit yields, and that by stocking at 3 hives per acre with bumble bees, or 5.1 hives per acre, almost twice as many berries can be produced.

## **Acknowledgements**

Thank you to the many blueberry growers and beekeepers involved in this study: we appreciate access to your fields and hives. Thank you also to Maurice Basque (New Brunswick Department of Agriculture - Tracadie office) for help in field selection and grower networking.

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Prepared by Robyn McCallum, Cameron Menzies, Kathleen Glasgow, and Sawyer Olmstead, 2017.